PLANT PATHOLOGY

A RECORD OF CURRENT WORK ON PLANT DISEASES AND PESTS

Published quarterly for the
Ministry of Agriculture, Fisheries and Food
Plant Pathology Laboratory
Harpenden, Herts

VOLUME 8, No. 4
December 1959

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HER MAJESTY'S STATIONERY OFFICE
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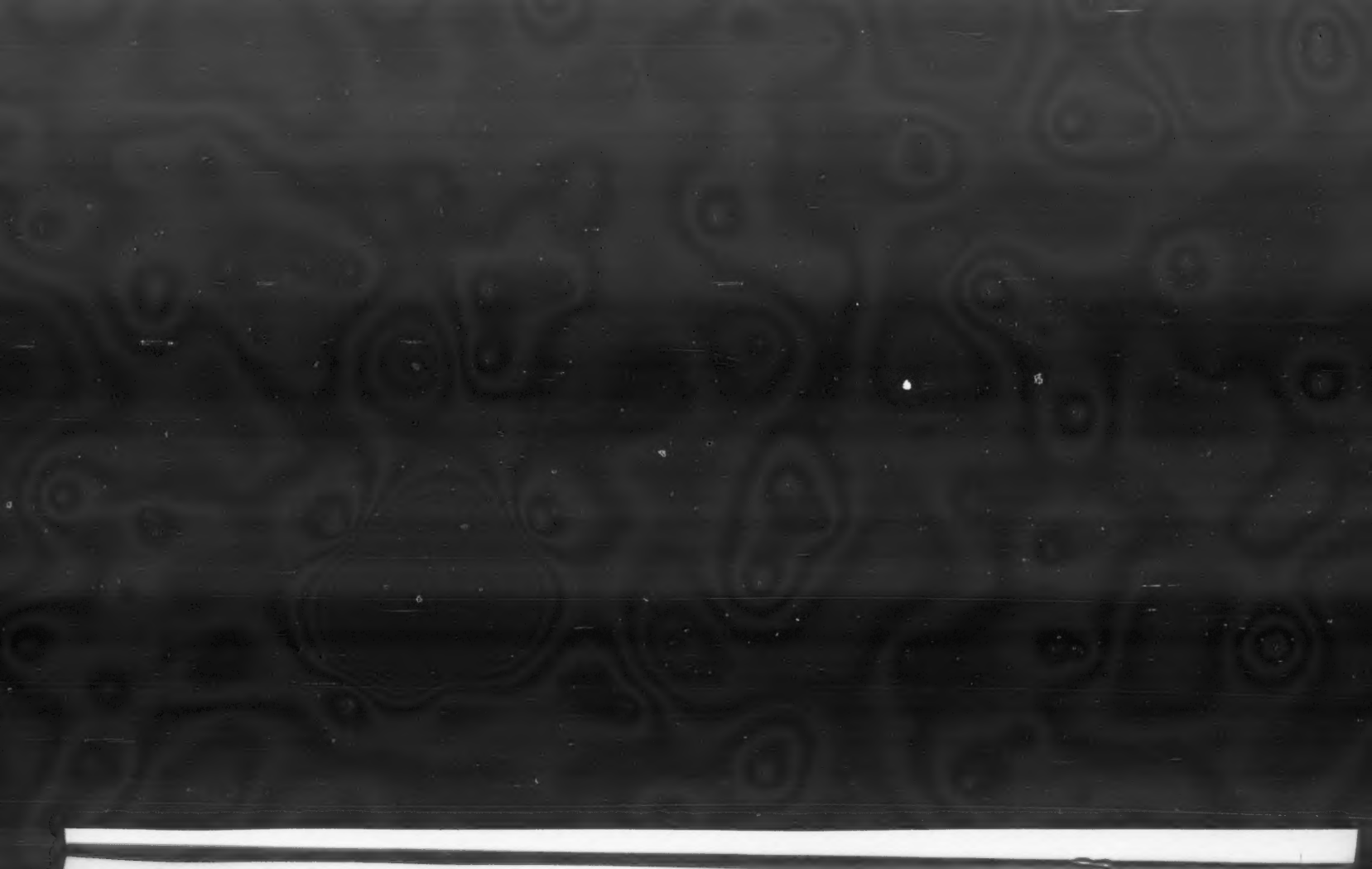

CONTENTS

Page

Trials of the Influence of Stem Builders on Bacterial Canker of Plum in the West Midlands. *Elizabeth R. Schofield and L. F. Clift* 115

The Phosdrin Content of Fresh and Deep-frozen Vegetables. *E. Q. Laws* . 121

A Broad Red Clover Resistant to Stem Eelworm. *E. B. Brown* . . 124

A Spot-sampling Method for the Estimation of Common Scab on Potato Tubers. *P. H. Lowings and W. J. Ridgman* 125

A Staining Method for the Study of Apple Scab Infections. *T. F. Preece* . 127

Some Records of Root Lesion Eelworms, *Pratylenchus* spp., in England. *J. F. Southey* 130

Nicotiana clevelandii Gray as a Test Plant for Plant Viruses. *M. Hollings* . 133

Frit Fly in Spring Oats. III. Sampling for Second Generation Grain Attack 137

The Susceptibility of Atle Wheat to Attack by the Wheat Blossom Midges. *H. F. Barnes, B. S. Miller and Margaret K. Arnold* 143

Sugar Beet Yellows in Great Britain, 1958. *R. Hull* 145

Soil Sampling Procedures for Potato Root Eelworm Cysts. *B. M. Church, H. C. Gough and J. F. Southey* 146

New or Uncommon Plant Diseases and Pests 151

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TRIALS OF THE INFLUENCE OF STEM BUILDERS ON BACTERIAL CANKER OF PLUM IN THE WEST MIDLANDS

by ELIZABETH R. SCHOFIELD
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IN 1951, about one-third of the plum trees in England and Wales, amounting to some 1¾ millions, were in the West Midland Region. Victoria and Pershore Yellow Egg are the principal varieties, but others widely grown include Purple Pershore, Czar, Rivers' Early Prolific, Marjorie's Seedling, Burbank's Giant Prune and Belle de Louvain. Of these, Czar, Victoria and Burbank's Giant Prune are especially susceptible to bacterial canker.

Despite the great susceptibility of Victoria to bacterial canker and silver leaf—the two most important diseases of plum—the market popularity of the variety has led to steadily increased plantings over the past twenty to thirty years. In 1944, Victoria formed 16 per cent of the West Midland's total of plum trees, and this had risen to nearly 18 per cent by 1951. The continued popularity of Victoria, leading to wide-scale planting, has meant that for many years there has been a high population of young trees of this variety. From the 1944 and 1951 censuses it can be estimated that, in the West Midlands in 1951, there were approximately 88,000 Victoria trees under the age of twelve years.

It is common experience that plum trees of over twelve years are rarely killed by bacterial canker, although cases of progressive losses in orchards of greater age are known. The preponderance of young Victoria trees in recent years would account for the frequency with which cases of bacterial canker causing death have been brought to the notice of advisory officers in Worcestershire, and in other plum-growing areas of the West Midlands.

Bacterial canker attacks have always been sporadic, unpredictable and varying in intensity. When the disease is severe large cankers occur and, if these girdle the main stem, the tree is doomed. Where the disease is less severe only a branch or two may be lost, or a relatively small stem canker may occur that heals over to leave only a scar.

It has been shown that bacterial canker of cherry may be reduced considerably by spraying the trees with Bordeaux mixture (Moore, 1946), but the same is not true of the disease on plum. In recent trials at Luddington Experimental Horticulture Station, and in earlier trials elsewhere (Moore, 1946; Montgomery, Moore and Hoblyn, 1943), no control of the canker phase of the disease resulted from Bordeaux spraying. Wormald (1932) suggested that one possible method of controlling bacterial canker in susceptible varieties of plum might be by the use of resistant varieties to build the stem of the tree. He later described trials (Wormald, 1934), in which a lower percentage of Victoria trees were killed when worked high on Myrobalan B or Common Plum, than when worked low on these varieties. In these trials the framework of the tree was produced by the scion variety. In subsequent trials at East Malling Research Station (Montgomery, Moore and Hoblyn, 1943), the use of resistant varieties to form both the stem and crotch of trees, on which were grafted the susceptible variety Victoria, proved a highly successful method of preventing the occurrence of stem cankers.

At the request of the West Midland N.A.A.S. horticultural officers, it was decided to carry out trials to determine the effect of stem-builders on the incidence of bacterial canker of plum under West Midland conditions. In 1948, a sub-committee of the Fruit Group, consisting of Dr. H. E. Croxall and Mr. L. F. Clift of the West Midland N.A.A.S., Mr. R. W. Marsh of Long Ashton Research Station, and Dr. H. B. S. Montgomery of East Malling Research Station, met to consider plans for the trials. It was agreed that the two canker-susceptible varieties, Victoria and Burbank's Giant Prune, should be worked on Pershore Yellow Egg, Warwickshire Drooper and Myrobalan B respectively. It was further agreed that half of each of the scions should be budded at the usual few inches above ground level and the other half high-worked on a framework of the rootstock variety.

Pershore Yellow Egg rootstock was chosen because of its local popularity, although it was not thought to be especially resistant to bacterial canker. Warwickshire Drooper, also used locally as a rootstock, had the reputation of being resistant. Myrobalan B was known to have high resistance.

MATERIALS AND METHODS

Two hundred rootstocks of each of the three varieties Myrobalan B, Warwickshire Drooper and Pershore Yellow Egg were planted in the winter of 1948–49 on a commercial nursery in Worcestershire. In the following spring only 25 per cent of the Pershore Yellow Egg were found to be true and many died, necessitating replacement of all the rootstocks in the winter of 1949–50.*

One hundred of each of the rootstocks were budded at the usual height in July 1950, and cut back to the bud in spring 1951. The resulting maidens were headed at three feet six inches in spring 1952. The primary branches were pruned in spring 1953 and the trees, with two-year heads, were available for transplanting in the autumn of that year. The remaining hundred stocks of each variety were cut back to the ground in 1951, thus giving stems of the same age as the high-worked stocks. The maidens were headed back to three feet six inches in spring 1952 and the primary branches grafted in spring 1953 giving, in effect, a two-year head in the autumn of 1953 ready for transplanting.

Trials were at three centres: Pershore Institute of Horticulture; Bank Farm, Mathon, Nr. Colwall; and Luddington Experimental Horticulture Station. The three trials were all in cultivated orchards and had a common object, but the treatments, layouts and possible observations were different.

TRIAL AT PERSHORE

The trial consisted of four treatments with each of the two scion varieties, Victoria and Giant Prune, arranged in two adjacent blocks. The four treatments were high- and low-working of the scion variety on Pershore Yellow Egg and Myrobalan B rootstocks. These were randomized and replicated three times within each varietal block. Each treatment consisted of eighteen trees planted eighteen feet apart in each direction in the winter of 1953–54.

Owing to unfavourable growing conditions, many trees died during 1955 and the trial was abandoned in the spring of 1956. In 1954, only three trees showed bacterial canker lesions; two of these were Giant Prune, low-worked on Myrobalan B and Pershore Yellow Egg respectively; the third was Victoria, low-worked on Pershore Yellow Egg.

* Clonal material of Pershore Yellow Egg and Warwickshire Drooper was not available.

TRIAL AT MATHON

The trial was in an enclosed valley, at an altitude of about 500 feet, near the foot of the west face of the Malvern Hills. The valley runs in a north to south direction, and is bounded on its west side by a bank bearing an orchard of plums with a history of bacterial canker.

The scion variety was Victoria, and this was both high- and low-worked on the three rootstocks Myrobalan B, Pershore Yellow Egg and Warwickshire Drooper, giving a total of six treatments. Each treatment was replicated four times and comprised twenty-four trees. The trial was laid out in four randomized blocks each containing the six treatments. The design was provided by the Statistical Department of East Malling Research Station.

The trees were planted, twenty feet apart in each direction, in the winter of 1953–54. The trial was concluded in 1957. Detailed tree-by-tree observations were made yearly in late summer or early autumn. On these occasions, records of disease incidence, tree damage and general information on tree growth and vigour were taken.

In 1954, about one-third of the trees showed implement damage which was mostly slight, but occasionally severe; in addition, seven showed damage by cattle. By 1955 most of this damage had healed satisfactorily, but the stakes were causing further injury to some trees. Little unhealed mechanical damage was observed in 1956 or 1957, but by 1957 the ties were beginning to cause constriction to the stems. Growth throughout was vigorous.

TABLE 1

Mathon Trial: Numbers of Victoria Trees in 24 Affected and not Affected with Bacterial Canker, 1954–57

		Rootstocks						Difference Required for Significance	
		Myrobalan B		Pershore Yellow Egg		Warwickshire Drooper			
		High	*Low*	*High*	*Low*	*High*	*Low*	*5 per cent*	*1 per cent*
Total trees infected with bacterial canker	1954	0	0	0	0	0	2		
	1955	2	14	7	16	4	9		
	1956	3	18	13	19	8	13		
	1957	5	22	21	21	11	15	5·7	7·9
Total trees killed by bacterial canker	1954	0	0	0	0	0	0		
	1955	0	12	5	14	3	7		
	1956	0	14	10	14	4	8		
	1957	3	21	17	21	9	14	6·1	8·5
Total healthy trees	1954	24	24	24	24	24	22		
	1955	22	10	17	8	20	15		
	1956	21	6	11	5	16	11		
	1957	19	2	3	3	13	9	5·7	7·9

In each year trees either dead or dying from bacterial canker were recorded separately from those showing only slight or moderate infection of the wood. The records for the four years are summarized in Table 1. It is noteworthy that bacterial canker rarely affected the wounds, and that bacterial shot-holing was prevalent in each year.

Records of bacterial canker were, in a few instances, complicated by the occurrence of symptoms that were more typical of infection with bark split virus than of bacterial canker. In Table 1 only trees with symptoms typical of bacterial canker infection are recorded. In the course of the trial, ten trees were observed with symptoms suggestive of infection with bark split virus. Six of these were on Myrobalan B rootstocks, two on Pershore Yellow Egg and two on Warwickshire Drooper. Five trees were observed with symptoms of silver leaf; one died in the first year and was replaced.

TRIAL AT LUDDINGTON

The trial at Luddington was laid out similarly to that at Mathon, using the same six treatments, but with Giant Prune as the scion variety. The treatments were replicated six times and each consisted of twenty-four trees. The trees were planted in the winter of 1953–54 on an exposed site adjacent to a variety trial in which bacterial canker had been prevalent. Growth generally was good although weak branches have been recorded on a few high-worked trees.

This was the only trial of the three in which detailed growth and cropping records were possible, and observations are continuing. Both sets of records are taken yearly and include the height and spread of each tree, the girth measured at twelve inches from the cut or union, and the yield of fruit in lb per tree. Detailed growth and yield records for each of the first six years of the trial will be published elsewhere. The growth measurements in 1958 and the total mean yields per tree for the period 1955–58 inclusive are given in Table 2.

TABLE 2

Luddington Trial: Growth Records for 1958 and Yields for 1955–58

	Rootstocks					
	Myrobalan B		Pershore Yellow Egg		Warwickshire Drooper	
	High	*Low*	*High*	*Low*	*High*	*Low*
Mean tree girth in inches.. ..	8·3	8·5	5·4	5·5	6·3	6·3
Mean tree height in feet	10·5	10·5	7·9	7·9	8·2	8·5
Mean tree spread in feet	7·2	6·9	5·2	5·2	5·9	6·5
Total mean yields per tree in lb 1955–58	54	50	34	37	39	54

As at Mathon, a considerable degree of mechanical damage occurred during the first six years of the trial. In most instances the wounds healed satisfactorily, and did not become the site of bacterial canker infections.

Although in all seasons bacterial leaf-spotting and shot-holing was prevalent, the incidence of stem cankers was slight and, up to 1958, only three trees died. All three were low-worked, one on each of the rootstocks. Besides these, bacterial canker was observed on eight low-worked trees; five of these were on Myrobalan B rootstock and three were on Pershore Yellow Egg. Bacterial cankers have not so far been observed on any high-worked trees. As at Mathon,

the recording of bacterial canker has been complicated by the occurrence, in some trees, of symptoms more like those caused by infection with bark split virus than by bacterial canker.

DISCUSSION

In all three trials bacterial leaf-spotting and shot-holing have been prevalent each year. Both at Luddington and at Mathon tree growth has been good and the trials were close to plum plantations in which canker is severe. It is only in the Mathon trial, however, that the incidence of stem cankers has been high, and it is from this trial that the main conclusions can be drawn. This difference in the incidence of cankers may be one of environment. In contrast to the prevailing exposed conditions at Luddington, the Mathon trial, although at an altitude of about 500 feet, was set in a sheltered valley with relatively high rainfall.

The performance of the trees high-worked on Myrobalan B was outstanding. Whether the total number of trees infected or the total number killed is considered, high-worked Myrobalan B showed a significantly greater resistance, at the 1 per cent probability level, than any of the low-worked trees. At the 1 per cent level also it showed significantly greater resistance than high-worked Pershore Yellow Egg. Compared with high-worked Warwickshire Drooper, the difference is only significant at the 5 per cent probability level.

Pershore Yellow Egg showed no resistance to the disease, there being only three healthy trees, low-worked and high-worked, at the end of the trial. This is somewhat surprising as, in Worcestershire, few complaints are received about this variety. It must be remembered, however, that unlike Victoria, it has not been extensively replanted in recent years.

The performance of Warwickshire Drooper was intermediate between that of Myrobalan B and Pershore Yellow Egg. The interesting feature about this variety, when employed as a rootstock, is that the difference in the degree of infection between high- and low-worked trees just fails to be significant. In addition, both high- and low-worked trees on this stock were significantly better, at the 5 per cent level, than low-worked trees on either Myrobalan B or Pershore Yellow Egg. At Luddington also, only one tree low-worked on Warwickshire Drooper was infected, compared with six on Myrobalan B and four on Yellow Egg. This would suggest that the resistance of the stock has been to some extent transferred to the scion. Too much reliance cannot be placed on this observation without further investigation. It has been suggested by Moore (1946), however, that some of the Mazzard rootstocks are capable of inducing a greater resistance in some varieties of cherry.

Although valuable as a stock, owing to its resistance to bacterial canker, Myrobalan B has two major disadvantages. Firstly, unless the scion variety is like Giant Prune in needing a strong stock to produce sufficient growth, it produces an undesirably large tree, especially on strong soil. Secondly, the variety has a strong tendency to persistent sucker production on the stem, despite the annual removal of such shoots. A valuable contribution to the control of bacterial canker would be the introduction of the resistant qualities of Myrobalan B into a more manageable rootstock by breeding. Warwickshire Drooper on the other hand, although not so resistant as Myrobalan B, has the merit of not suckering to any extent. Clones of this stock, however, are not available and the type is subject to variation. In this trial, moreover, the "take" of buds and grafts was not good on this variety and, if this is common experience, the stock is not likely to be popular with nurserymen.

Although effective, the method used in these trials to produce high-worked trees was both lengthy and expensive. The staddle method of working is generally

recommended for cherries, but with this crop the bacterial canker problem is rather different from that on plum. It is the crotch of the tree that frequently becomes infected in cherries, and tree death is less likely if it can be constructed of resistant wood. For high-worked plum trees it has been suggested that a single graft or bud is all that is necessary, but evidence on this point is limited.

SUMMARY

Three trials are described in which the two canker-susceptible varieties, Victoria and Burbank's Giant Prune, were each high- and low-worked on the three rootstocks Warwickshire Drooper, Myrobalan B and Pershore Yellow Egg. In the one trial where severe infection occurred, Pershore Yellow Egg showed little resistance to canker when employed as a stem-builder. Warwickshire Drooper showed intermediate resistance, and the high degree of resistance of Myrobalan B, observed by other workers, was again demonstrated. There was an indication that Warwickshire Drooper induces some degree of resistance in Victoria, but this observation needs further investigation. Although the value of Myrobalan B as a stock on which to top-work a susceptible variety is clearly shown, the variety has two major disadvantages: persistent sucker production and the liability to produce a large tree, especially on strong soil.

We thank Mr. Stephen Ballard, the Pershore Institute of Horticulture and Luddington Experimental Horticulture Station for the provision of trial facilities; Dr. H. E. Croxall for the statistical analysis of the results, and members of the staff at Luddington for their help in the recording.

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AMERICAN PHYTOPATHOLOGICAL SOCIETY

The Golden Jubilee volume of this Society's Proceedings, entitled *Plant Pathology, Problems and Progress, 1908–1958*, was published in June 1959 by the University of Wisconsin Press, 811 State Street, Madison, Wisconsin, U.S.A. Price $8.50. The volume contains 588 pages, 4to, with 32 Plates, 39 text-figures, and a full analytical index.

THE PHOSDRIN CONTENT OF FRESH AND DEEP-FROZEN VEGETABLES

by E. Q. LAWS

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WHEN vegetables treated with a systemic phosphorus insecticide are stored in a domestic refrigerator the break-down of the insecticide continues as it would at ordinary temperatures. In the case of phosdrin, which is quickly destroyed by the plant enzyme system, the interval between sampling and analysis must clearly be short if the results are to have a meaning. Consequently, it is a matter of interest to the analyst that samples for examination should be capable of being stored for long periods without alteration of the insecticide content. Deep-freeze refrigeration appeared to offer the best method of storage. It was for the purpose of studying the long-term effects of deep freeze on the insecticide content of treated vegetables that the present work was undertaken. The further implication of the work is that if the deep freeze totally inhibits the plant enzyme system, then the insecticide will be present in the stored product at the same concentration as it was in the plant at the time of harvesting, subject only to alterations of moisture content. The results show that, in general, the insecticide is preserved in the deep-frozen samples. This outcome of the experiments is satisfactory to the analyst, but it raises problems for those concerned in the commercial preservation of vegetables by this process.

The vegetables available in October 1958, when these experiments were begun, were kale and sugar beet. Although it was not the usual time of year for insecticide application, the results do not appear to be influenced by this factor. Phosdrin was applied on October 21, 1958, at the rate of 4 oz of active ingredient per acre, by hand spraying and with the addition of a wetting agent. Representative samples were obtained by hand picking selected leaves from the plants under test. Control samples of untreated crops were taken at the same time.

METHODS OF ANALYSIS

Chemical. The method of Laws and Webley (1959) for the determination of demeton-methyl was used. This employs a chromatographic separation of the insecticide from interfering plant substances, followed by a photometric measurement of the phosphoric acid content of the separated insecticide. One modification was introduced; a reflux condenser was fitted to the flask in the early stages of the wet oxidation to prevent loss of insecticide by volatilization.

Cholinesterase Inhibition. The method of Hensel, Hewitt, Sheets and Scott (1954), as modified by Boyd (1957) was used. This method utilizes the measurement of the pH change of a buffered blood plasma to determine the insecticide content, by evaluation of the percentage inhibition of the enzyme in terms of a known amount of the pure insecticide. The determinations were made on chloroform extracts obtained by the same process as for the chemical method, but not in all cases on the same portions of the samples.

The results obtained are set out in Tables 1 and 2 for kale and beet respectively, and are expressed as parts per million on the sample as it stands. They represent values obtained by both methods at the time of sampling, and after approximately six weeks and six months of deep-freeze storage. In most cases only a single determination was possible for each sample at a given time, but where more than one result is shown they were obtained by separate experiments on different portions of the stored vegetables.

TABLE 1

Kale Sprayed with Phosdrin at the Rate of 4 oz per acre
Residual Phosdrin as p.p.m. on Sample

Sample No.	Time from Spraying to Sampling *Hours*	Chemical Determination by Phosphorus Content			Anticholinesterase Measurement		
		At Time of Sampling	*After Storage in Deep Freeze for:*		*At Time of Sampling*	*After Storage in Deep Freeze for:*	
			6 weeks	*6 months*		*6 weeks*	*6 months*
1 ..	1½	5·8	3·2	4·2, 7·1	6·7	1·4	3·0
2 ..	4½	4·2	5·3	3·4, 4·2	6·0	9·0	3·9, 7·3
3 ..	21	2·0	1·9	1·9, 3·5	1·7	4·3	—
4 ..	26	1·6	2·0	2·3, 1·9	1·8	—	0·9
5 ..	44½	1·4	2·0	3·2, 2·9	—	0·6	2·8, 2·9
6 ..	49½	2·2	2·4	3·0, 2·9	1·8	—	3·1, 2·5
7 ..	68½	1·1	1·1	0·8, 1·2	1·1	0·6	1·3
8 ..	168	0·6	—	—	0·6, 0·8	—	—
Control		0·09	The control sample value has been taken into account in the figures quoted.				

TABLE 2

Sugar Beet Tops Sprayed with Phosdrin at the Rate of 4 oz per acre
Residual Phosdrin as p.p.m. on Sample

Sample No.	Time from Spraying to Sampling *Hours*	Chemical Determination by Phosphorus Content			Anticholinesterase Measurement		
		At Time of Sampling	*After Storage in Deep Freeze for:*		*At Time of Sampling*	*After Storage in Deep Freeze for:*	
			6 weeks	*6 months*		*6 weeks*	*6 months*
1 ..	1½	3·7	6·4	4·5, 3·9	5·8	2·0	6·5, 6·0
2 ..	4½	3·6	3·8	3·6, 3·5	5·5	3·9	2·2, 1·9
3 ..	21	1·1	1·3	2·8	1·1	1·0	1·0
4 ..	26	1·0	1·3	1·3	0·9	1·0	0·3
5 ..	44½	1·3	0·9	1·6	—	0·3	0·4
6 ..	49½	1·4	0·7	1·2	0·9	—	0·2
7 ..	68½	1·0	1·4	1·0	0·2	0·1	0·4
8 ..	168	0·4	—	0·4	—	—	—
Control		0·08	The control sample value has been taken into account in the figures quoted.				

DISCUSSION

The figures obtained are not uniformly consistent, and are occasionally anomalous, and it is difficult to ascertain the causes of the discrepancies. The divergence of values found in the early samples by both methods may well be

due to spray on the surface of the vegetables giving rise to local pockets of insecticide, which would not be present on the later ones, as the insecticide would by that time be wholly within the plant. Apparent increases in insecticide content on storage may be due to the same cause, or they may be the result of withdrawal of water by freeze drying of the vegetable matter; although this is unlikely as the samples were in polythene bags, and any liquid or ice was taken into account in selecting the sample. In the case of the sugar beet, the low results for sample 7 by anticholinesterase measurement, compared with the chemical method, might be due to selective decomposition of the biologically more active isomer of the phosdrin. The standard used was the mixture of the isomers in the form of the "commercially pure" product, and does not necessarily have the same proportion of the cis- and trans-isomers as the phosdrin which has been in the plant for some time.

The Tables show that the decay from the original high level of phosdrin can be followed in samples which are fresh, or in those which have been stored for six weeks or six months. The pattern of decay is similar in the two crops studied, and both methods show the presence of the insecticide in the vegetables. The chemical results appear to be more consistent, while the anticholinesterase figures demonstrate that the residues are biologically active. A statistical analysis of the results gives little further information. Some of the figures recorded here have appeared previously in *The Analyst* (Laws, 1959); the remainder have not been published before.

SUMMARY

Phosdrin residues in samples of kale and sugar beet leaves collected at intervals from 1½ to 168 hours after spraying were determined at the time of sampling, and after storage of the material for six weeks and for six months in deep freeze. Both chemical and cholinesterase-inhibition methods of determination were used. The results indicate that the residues after storage in deep freeze are of the same order as those in the fresh material, and that the diminution of phosdrin in the leaves from 6–7 p.p.m. 1½ hours after spraying to less than 1 p.p.m. after 168 hours can be followed in the fresh or deep-frozen material.

I thank all who have helped in this work, and in particular those at Rothamsted Experimental Station and the Plant Pathology Laboratory, Harpenden, who provided the treated crops and control samples, and my colleagues who carried out the analyses.

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A BROAD RED CLOVER RESISTANT TO STEM EELWORM

by E. B. BROWN

National Agricultural Advisory Service, Cambridge

IN 1955, a field in Hertfordshire was visited, on which two stocks of broad red clover had been sown the previous year. One of these stocks was severely attacked by stem eelworm, *Ditylenchus dipsaci* (Kühn) Filipjev, and the other, drilled a day later, was unaffected. The division between these two stocks was not absolutely distinct, but consisted of a drill-width of mixed good and poor plants, showing how there had been some mixing of the two stocks in the drill. Small numbers of infested plants could be found up to five yards into the good part but no evidence of even a slight attack could be found on the remainder. The only logical explanation of this difference appeared to be that the good clover stock (A) was resistant to stem eelworm attack, and the farmer kindly agreed to harvest this part separately.

A quantity of this seed was obtained for further trial. Its performance was compared, in pots, with two other stocks of broad red clover (B and C), by inoculating four pots of each with about 1,000 eelworms from one source, similarly with eelworms from another source, leaving four pots of each to which no eelworms were added. By early March the following year all the inoculated pots in the two stocks (B and C) had either failed completely or contained only severely attacked plants. The eight inoculated pots of the good stock (A) were uniformly good and there was no visual difference between these and the uninoculated pots.

A more critical examination was made of the clover plants in two of the inoculated pots of stock A. In one, twelve plants were uninfested and one contained a very few eelworms and eggs. In the other, twenty plants were uninfested, three with very slight symptoms contained very few eelworms and eggs, one with moderate symptoms also contained a few eelworms and eggs, and one with fairly severe symptoms contained moderate numbers of eelworms and eggs.

The experiment has confirmed that this stock A has a remarkable degree of resistance, even though there may have been a slight amount of cross-fertilization in the field, and also a possibility that a small quantity of the poor stock was harvested with it. Even the numbers of eelworms in the plant with fairly severe symptoms were much lower than would normally be found in a heavily-attacked plant, and the inoculum of 1,000 eelworms per pot is probably very much higher than a clover plant would encounter in the field.

The stock was found entirely by chance. It may not be the only resistant stock in use in Great Britain and there may well be others with varying degrees of resistance. It is now undergoing further tests at both the Plant Breeding Institute and the National Institute of Agricultural Botany at Cambridge.

I am indebted to Mr. R. P. Hawkins of the N.I.A.B. for confirming that this clover was a broad red and not a single cut or late-flowering type.

A SPOT-SAMPLING METHOD FOR THE ESTIMATION OF COMMON SCAB ON POTATO TUBERS

by P. H. LOWINGS *and* W. J. RIDGMAN

School of Agriculture, Cambridge University

IN a sample of potato tubers the proportion of the surface attacked by common scab, *Streptomyces scabies*, is commonly estimated by eye, with reference to a standard key (Clark, Stevenson and Schaal, 1938; Large and Honey, 1955). While this technique is valuable for surveys, its application in experimental work is limited by the breadth of the disease categories into which the tubers must be placed, which may conceal small but important effects of treatment. There may also be a personal factor involved in an estimate of this type (Stevenson, Schaal, Clark and Akeley, 1942), and this may become important in long-term field experiments where it may be unavoidable that different individuals should carry out the scoring in different seasons. We have, therefore, attempted to overcome some of these limitations by developing a more objective sampling technique based on the principle of the point quadrat method of determining botanical composition of grassland (Brown, 1954).

A strip of transparent, flexible plastic was marked centrally with a longitudinal line of equidistantly-spaced circular dots. The dots, which in our strips were 1 mm in diameter and 8 mm apart, were numbered consecutively from a transverse line indicating "zero" which was drawn near one end of the strip. We found it convenient to type the numbers on to the strip, but the dots were made with photographers' red opaque ink, the whole strip being protected by adhesive plastic tape.

To estimate the proportion of the surface area attacked by scab in a bulk of potato tubers a random sample is scored by looping the plastic strip tightly round the long axis of each tuber in turn, as though measuring it with a tape measure, and noting the figure marked against the dot falling nearest the "zero" line (that is, the total number of dots round the tuber's long axis), and also recording the number of dots falling on scab lesions. The latter figure may then be expressed as a percentage of the former to give an estimate of the amount of scab around the long axis and also, if scab is randomly distributed over the tuber surface, an estimate of the percentage of the total area covered by scab.

The accuracy of the technique was tested in two ways. First, to determine whether scab was randomly distributed over the tuber surface, a sample of one hundred scabbed Majestic tubers was scored twice, by taking one reading about the long axis and one about the short axis of each tuber. There was no significant difference between the results. Secondly, a direct comparison was made between the results obtained with the spot sampling technique and those resulting from direct measurements of the percentage of scabbed area on the same tubers. To do this twenty-one tubers were selected to represent a range from approximately three per cent up to twenty-five per cent surface covered with lesions. The amount of scab on each tuber was first estimated by a single score with the spot sampling technique, and then the actual area of scab was obtained by measuring the mean diameter of each scab lesion with dividers and calculating its area. The total area of each tuber was calculated by measuring the ends and the central portion separately. The ends were cut off, peeled, the outlines of the peel drawn

on squared paper and their area determined. This was then added to the area of skin on the central portion of the tuber, which was calculated by treating it as a cylinder and measuring its length and mean diameter. A very highly significant correlation ($r = 0{\cdot}89$ with 20 degrees of freedom) was found between the percentage of area covered by scab estimated by spot sampling and the percentage calculated from direct measurement. These measurements are given in summary form in the Table.

Comparison of Direct Measurement and Spot Sampling Estimates of Tuber Area Covered by Scab. Variety: Majestic

Tubers Grouped by Direct Measurement of Area Covered by Scab		Mean Percentage Area Covered by Scab. Measured by:	
Area of Scab per cent	*Number of Tubers in Group*	*Direct Measurement*	*Spot Sampling*
< 5·0	8	3·70	4·10
5·1–10·0	8	7·00	7·79
>10·0	5	14·63	18·64

With a technique of this type, the number of tubers which should be examined to give results of a specified accuracy will depend on the amount of scab present, and on the variability within the sample. Furthermore, as the spots on the sampling strip are necessarily of a finite size, while they should, for perfection, be infinitely small, the results obtained by this technique will normally indicate a slightly higher percentage of scab than is actually present. The extent of this error will depend on the size of the spots used (Warren-Wilson, 1959). For experimental purposes, where comparative rather than absolute measurements are required, it would rarely be necessary to discover a correction factor as the error should be consistent so long as a standard form of spot sampling is used throughout.

We wish to thank Mrs. E. L. Whitby for her technical assistance with this work.

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A STAINING METHOD FOR THE STUDY OF APPLE SCAB INFECTIONS

by T. F. PREECE

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IN recent years an improved method has been in use for the detection of fungus infection in man (Kligman, Mescon and De Lamater, 1951; Sharvill, 1952). The material is treated with periodic acid, followed by staining with decolorized basic fuchsin. The fungus stains a bright red, leaving the host tissue colourless. The ease with which fungus material can be detected by this technique is a dramatic improvement on the hitherto usual method of examining unstained, potash-cleared specimens. I therefore tried the method on plant material, in particular on apple leaves bearing lesions of *Venturia inaequalis* (Cooke) Wint. Work in progress on the infection of apple trees by artificial inoculation provided the material. A description of the method as used on entire leaves is now given, with some illustrations of the results obtained.

It was found that the method of staining was applicable to freshly collected green leaves; to leaves preserved in formal-acet-alcohol; to leaves after extraction of the green pigments with methyl alcohol—with or without further clearing in a saturated aqueous solution of chloral hydrate; and to epidermal layers of the leaves removed by treatment with oxalate solutions. The staining procedure, after rinsing in distilled water, was as follows:

1. Immerse in 1 per cent periodic acid for 5 minutes.
2. Wash in distilled water for 5 minutes.
3. Immerse in decolorized basic fuchsin for 5 minutes.
4. Wash in sulphurous acid solution for 5 minutes.
5. Immerse in distilled water for 5 minutes.
6. Mount in distilled water for examination.

The washing in water after the sulphurous acid is an important part of the procedure, as much of the final colour develops at that stage. The stained material can be preserved for some time in ethyl or methyl alcohol without loss of colour. The reagents are prepared as follows:

PERIODIC ACID. A freshly-prepared 1 per cent solution in distilled water.

DECOLORIZED BASIC FUCHSIN. 1g basic fuchsin is dissolved in 200 ml distilled water by boiling. Cool, and add 20 ml N/1 HCl. Mix well and add 1g potassium metabisulphite crystals, shaking until dissoved. Plug the flask and leave overnight in the dark. The solution should be straw-coloured; if it is too pink, add 2g powdered charcoal, and filter immediately. Store the solution in a dark bottle with a tight stopper. The reagent thus prepared should keep satisfactorily for about one month.

SULPHUROUS ACID. To 200 ml distilled water add 10 ml of a 10 per cent solution of potassium metabisulphite crystals in distilled water and 10 ml of N/1 HCl. Prepare just before use and discard any unused solution.

On freshly-picked green leaves and fruit, stained directly without removal of pigments, there is an excellent contrast in colour between the red-stained lesions and the green background, both to the naked eye and under the microscope. The distribution pattern of infection is clearly revealed, and lesions can be detected in the field at a very early stage. This is of importance in relating infections to periods of leaf wetness and other meteorological data.

Clearing of the leaves by removal of the green leaf pigments is necessary for photography. Preservation of the material in formal-acet-alcohol fixative before staining effects a partial clearing, but leaves the tissues a yellow-brown colour unsuitable for photography. The best results were obtained by steeping the

freshly collected leaves in cold methyl alcohol (99 per cent technical), which generally removed most of the leaf pigments in two hours, and gave complete decolorization on standing overnight. Plate III, 1 shows a pair of artificially infected apple leaves, photographed with a green filter, before and after clearing and staining. Plate III, 2 shows a naturally infected leaf similarly treated. Plate III, 3 shows the detail of one of the dendritic lesions revealed at low magnification after staining.

GERMINATION AND INFECTION

Spores deposited on, or trapped by, the leaf itself in the orchard do not appear to be detached or disturbed by the clearing and staining processes. At the microscopic level the brilliant red coloration of spores and hyphae facilitates rapid searching of the leaf surface with a low-power objective. The growth from individual spores, and successive stages in the development of visible lesions, can then be studied and photographed with objectives of higher powers, using a green filter for contrast.

Plate II illustrates the use of the method in studies of the early stages of infection. Work in 1958 indicated that, of the susceptible new growth at the tip of an apple shoot, it is the youngest unrolled leaf that develops lesions most readily after inoculation. A standard volume (0·02 ml) of a freshly made suspension of conidia (500,000 per ml) was placed on the wetted upper surface of this leaf. A piece of wet cotton wool was wrapped round the petiole and a small polythene bag (2½ by 1½ in.) was placed over the leaf and closed by means of a piece of thin fuse wire. By this enclosure the leaf surface could be kept wet for any desired period in the orchard, and—in the absence of rain—the period of leaf wetness could be terminated simply by removal of the bag. Plate II, 1 shows a typical apple shoot with the test leaf bagged after inoculation. Conidia in a drop of the spore suspension used for inoculation, stained on a glass slide, are shown in Plate II, 2.

The part of the infection process revealed by removal, clearing and staining of inoculated leaves after various periods of bagging and subsequent exposure in the orchard is illustrated in Plate II, 3: (*a*) shows ungerminated conidia on the leaf surface one hour after inoculation and enclosure in the bag; (*b*) conidia beginning to germinate seven hours after inoculation, while the bag was still in place; (*c*) growth of germ-tubes after maintenance of eighteen hours of leaf wetness in the bag; and (*d*) similar growth after twenty-four hours of leaf wetness in the bag and nineteen hours subsequent exposure. The observations were made in June and July, when the mean temperature during the infection periods ranged from 65° F to 75° F. Plate II, 4 shows part of the surface of an inoculated leaf stained after an infection period, as seen on scanning at a lower magnification. The actual specimen had fourteen hours of leaf wetness in the bag and seven hours subsequent exposure. Similar groups of conidia could be found up to at least a week after inoculation, always with a wide range in the extent of growth of germ tubes, and always with a number of conidia apparently still ungerminated.

SUBCUTICULAR GROWTH

Mycelial growths similar to those shown in the classical drawings of Nusbaum and Keitt (1938) were seen and photographed occasionally; but even after staining for thirty minutes—which gives a very dense coloration of hyphae—it was difficult to decide how much of the growth was subcuticular, without resort to transverse sectioning of the leaf.

Some subcuticular growth revealed by staining a leaf *in toto* is shown in Plate II, 5, but such examples are hard to find. In general the subcuticular growth is stained poorly or not at all, but can be stained, however, if the leaf

epidermis is first removed. A method based on that of Martin, Batt and Roberts (1958) has been used for this purpose. After extraction of the leaf pigments in cold methyl alcohol, and removal of the wax from the cuticle by ethyl ether, the material is transferred to a 1·6 per cent solution of ammonium oxalate in 0·4 per cent oxalic acid. After the preparation has stood in this solution for about a week at room temperature, upper and lower epidermal layers can be readily peeled away from the rest of the leaf. Plate III, 4 shows part of a lesion on a piece of epidermis detached in this way, and photographed with a green filter, after staining.

OTHER APPLICATIONS

Although work has so far been confined to apple scab, it may well be that this staining method has a very much wider application in plant pathology. Germinating spores and superficial mycelium of a number of species of fungi have been observed in profusion on the surfaces of apple leaves taken from the orchard, and all this fungus growth has been stained as well as, or better than, that of apple scab. The staining is not entirely specific to fungus tissue. Chitin (Hotchkiss, 1948) is among the substances strongly stained; which suggests that the method may have useful applications in entomology. Leaf hairs (Plate II, 4), and cut or injured parts of the leaves, also take up some stain, but in general this does not lead to any confusion.

Preliminary experiments have indicated that the efficiency of the staining is not affected by phenyl mercury chloride, lime sulphur, copper oxychloride or captan preparations applied to the leaves for scab control. The effects of these materials on the morphology of germinating spores can clearly be seen on leaves stained after spraying.

Spores adhering to glass slides, after evaporation of the water from drops of spore suspension (Plate II, 2), and spores trapped on slides coated with vaseline, are stained equally well by this method. Kligman and Lewis (1953) have shown that outstandingly good stained preparations of fungus growth on artificial media can also be obtained.

SUMMARY

The periodic acid-Schiff staining method used in medical mycology has been found to be effective on plant material, particularly for the early detection of apple scab lesions. It enables the development of lesions from germinating spores to be studied and photographed on the surfaces of entire leaves.

I wish to thank Mr. E. C. Large for his interest in this work, and also for the photographs.

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SOME RECORDS OF ROOT LESION EELWORMS, *PRATYLENCHUS* SPP., IN ENGLAND

by J. F. SOUTHEY

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NEMATODES of the genus *Pratylenchus* are now regarded as important crop pests in several countries, but there are few published records of their occurrence in Britain. T. Goodey (1932) recorded them from roots of daffodil, lettuce, sweet pea and lawn grasses, Taylor found them in lily of the valley, Thompson in broad bean (Anon., 1936 and 1954), and Stone (1953) described an attack on delphinium. Pitcher (1957) has discussed their possible role in disorders affecting fruit trees in South-east England; he found that *P. penetrans* (Cobb) Sher and Allen was commonly present in the roots of such trees. That root lesion eelworms are common in British soils and in the roots of plants in this country is clear from unpublished reports sent by Advisory Entomologists and others to the Ministry of Agriculture's Plant Pathology Laboratory at Harpenden. These reports include records of *Pratylenchus*, sometimes associated with poor growth or root injury, from anemone, barley, broad bean, broccoli, chrysanthemum, clematis, fodder beet, iris, lily of the valley, mangold, mustard, narcissus, oats, potato, ryegrass, spinach, sweet corn, sweet pea, *Thalictrum*, wheat and white clover. The most frequently recorded host is narcissus in the south-west.

In most of the published and unpublished records, the nematodes were identified as *Pratylenchus pratensis* (*Anguillulina pratensis* or *Tylenchus pratensis* in earlier records) but, following Sher and Allen's (1953) revision of the genus, it is clear that *P. pratensis* as previously understood may have included several species. During the last few years specimens of various plants (mostly ornamentals from nurseries) received at the Plant Pathology Laboratory have shown root injury associated with parasitism by *Pratylenchus*, and opportunity has been taken to identify the species present.

TECHNIQUE

Infested roots were washed, cut into about $\frac{1}{4}$–$\frac{1}{2}$ inch lengths and treated for two minutes in about 250 cc water in an electric homogenizer. The resultant suspension was usually allowed to stand for 2–3 hours, and was then poured through a 24-mesh sieve (c. 1 mm aperture) to remove the larger pieces (mainly pieces of the hard vascular cylinder). The remainder, containing nematodes and finer root debris (mainly of cortical tissues), was either transferred direct to a Baermann funnel fitted with a cloth or paper-tissue filter, or the worms and debris were first collected by screening at least twice with a 250-mesh sieve. The method generally yielded good numbers of *Pratylenchus* for examination, but no attempt was made to assess its quantitative efficiency: the numbers obtained varied from 2 to 500 per gramme of root. Whenever possible, at least ten females and five or more males were measured and examined under an oil-immersion objective. Identification was mainly from the key and descriptions of Sher and Allen (1953). No evidence was obtained of mixed *Pratylenchus* populations in any of the plant material studied.

RESULTS OF OBSERVATIONS

The results of observations made on the plant material, and the nematodes recovered from it, are summarized as follows:

Host Plant	*Source and Sender*	*Species of* Pratylenchus	*Remarks*
Delphinium sp.	Nursery in Somerset (L. E. W. Stone)	*P. penetrans* (Cobb) Sher and Allen	Typical lesions and root rotting as described by Stone (1953)
Helleborus niger L.	Nursery in Lancashire (H. W. Thompson)	,,	Some typical lesions and severe root rotting
Clematis "Kermesina"†	Nursery in Hampshire (A. P. King)	,,	Typical dark-brown root lesions with some secondary rotting (see Plate I, 1)
C. vitalba L.* (seedling)		,,	
C. cirrhosa L.† (= *C. calycina*)	Nursery in Suffolk (T. Kingsley)	,,	
C. viticella L. "Little Nell"†		,,	
Ranunculus asiaticus L.*	Devon (H. G. Morgan)	,,	Many root lesions with secondary rotting; very high *Pratylenchus* population: 166 recovered from 0·3 g root
Anemone coronaria L.*	Cornwall (H. C. Woodville)	,,	Poor growth in patches corresponded with similar trouble in narcissus in previous years (see also below)
Narcissus "St. Agnes"	St. Mary's, Isles of Scilly (G. Baines)	,,	See below
Avena sativa L.	Farm in Hertfordshire	*P. pratensis* (de Man) Filipjev	No root lesions (see also below)
Pisum sativum L.	Devon (H. G. Morgan)	,,	Peas and adjoining broad beans showed yellowing and poor growth. No definite root lesions, but *Pratylenchus* abundant in both hosts. (The soil pH was found to be very low (3·9 in the worst area).)
Vicia faba L.		,,	

* Believed to be new host records for *P. penetrans*.

† The clematis species and varieties had been grafted on to *C. vitalba* root-stocks. It is understood that, after grafting and replanting, the scion may produce new roots as well as the stock but, since it was not possible to distinguish scion roots, it seems safest to assume that *C. vitalba* was the true host in these cases.

A few of the records tabulated above call for further comment.

Anemone. The roots of the specimens examined were so severely rotted that scarcely any remained intact and few *Pratylenchus* were recovered from them. However, the lower parts of the petioles and pedicels, i.e., those parts which had been between corm and soil surface, showed small, linear, brown lesions, many of which contained *Pratylenchus*. Soil from near the plants contained a high nematode population, including many Tylenchids (mainly *Rotylenchus* group—no *Pratylenchus* were seen), which might have contributed to root injury, as well as Dorylaimids, *Mononchus* spp. and other probably predatory forms. An unusual feature was the very large number of Tardigrada ("bear animalcules") recovered with the eelworms, some of which were also obtained from the root remains.

Narcissus. The narcissus bulbs, received in January 1958, had been taken from a holding where narcissus root rot, associated with *Pratylenchus*, was known to occur. The roots appeared fairly healthy at that time and showed only a few light-brown, streak-like lesions. After processing in the homogenizer, however, between 1,300 and 1,400 *Pratylenchus* were recovered from 16 g root. Narcissus root rot associated with the presence of root lesion eelworms and the fungus *Cylindrocarpon radicicola*, Wollenw., which is probably secondary, has caused concern in the Isles of Scilly and West Cornwall for many years (Anon., 1958). Slootweg (1956) has established that *P. penetrans* is a primary pathogen of narcissus roots and initiates a similar root rot in the Netherlands. Material examined by me previously, had always been of bulbs lifted later in the season

and showing severe root rot. In such material it was often difficult to find more than a few *Pratylenchus*. The present observations agree with the experience of Slootweg (1956) and of Morgan and Thomas (*in lit.*), that it is necessary to examine bulb roots early in the season, soon after active root growth has commenced, in order to find primary lesions and high *Pratylenchus* populations.

Oats. This case was brought to my notice by F. Widdowson of Rothamsted. The plants showed weak growth and yellowish striping of the leaves, and the main cause was thought to be manganese toxicity associated with low soil pH. The roots showed no obvious damage but numerous nematodes were recovered from them (over 1,500 from 20 g of roots): about 45 per cent were Tylenchids, of which almost half were *Pratylenchus; Rotylenchus* group came second in order of abundance. Discussion with colleagues has indicated that *Pratylenchus* is commonly found in cereal roots when making laboratory examinations, e.g., for *Heterodera major* O. Schmidt.

DISCUSSION

It is curious that all the host plants of *Pratylenchus penetrans* reported here, except narcissus, are members of the Ranunculaceae. There seems to be no suggestion in the literature (e.g., Oostenbrink, s'Jacob and Kuiper, 1957) that *P. penetrans* especially favours plants in that family, and it appears to have a very wide host range. Infestation was seen by me on another Ranunculaceous plant, *Thalictrum* sp., in Devon in 1948. The roots showed typical lesions and rotting and contained a high population of *Pratylenchus*, but suitable material has not been preserved for identification.

According to Seinhorst (1958), *P. penetrans* is by far the commonest species of *Pratylenchus* causing economic damage in the mainland of Western Europe; *P. pratensis* is very widespread, but does not generally cause definite symptoms and is of doubtful economic importance. It seems likely that the situation is similar in England. In all the cases where *P. penetrans* was found, it was associated with well-defined root lesions, as described by Stone (1953) and others, often followed by secondary rotting. On the other hand, none of the three plants from which *P. pratensis* was identified showed definite root symptoms, and there were alternative explanations for poor growth connected with soil conditions.

Thanks are due to all those mentioned in the Table for sending plant material and providing information.

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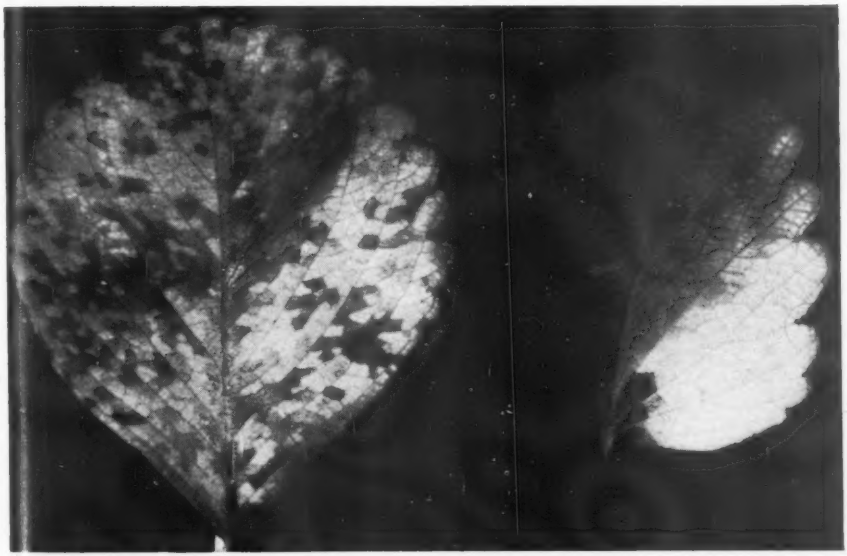
ROOT LESION EELWORMS

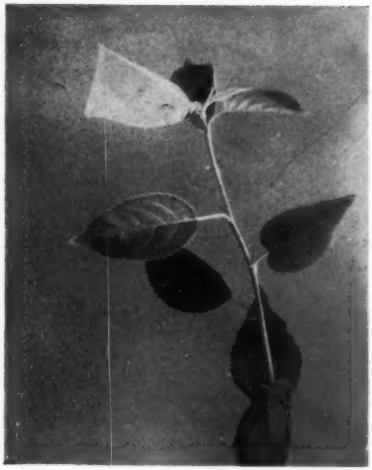
1. Clematis root showing lesions associated with *Pratylenchus penetrans*.

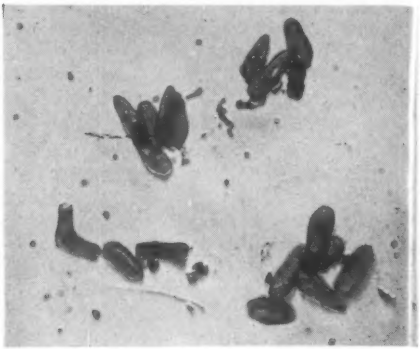
EELWORM ON STRAWBERRY (see p. 152)



2. Endoparasitic damage caused by *Aphelenchoides ritzema-bosi* to strawberry leaves.

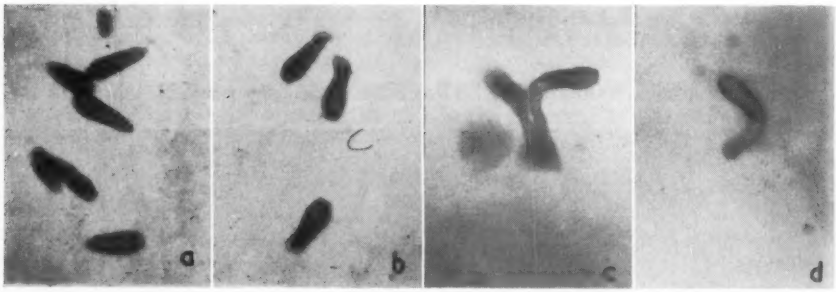
PLATE I



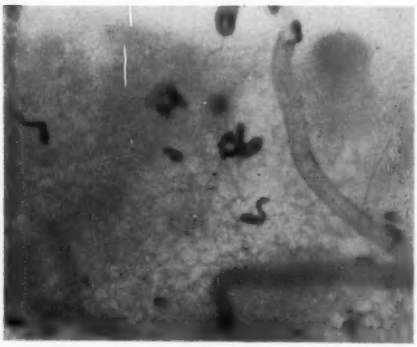


1. *Left:* Position of leaf inoculated and maintenance of surface wetness by polythene bag.

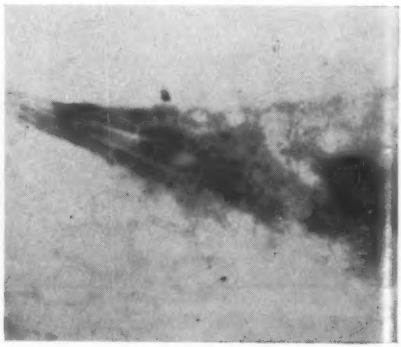
2. *Above:* Freshly collected conidia as used for inoculation, stained on a glass slide. × 420.



3. Germinating conidia on leaf surface, 1, 7, 18 and 43 hours after inoculation. For further details see text. × 420.

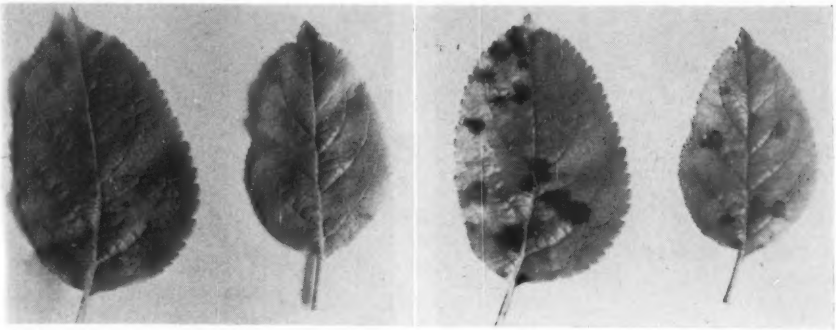


4. Part of leaf surface scanned after an infection period, showing stained conidia and leaf hairs. × 170.

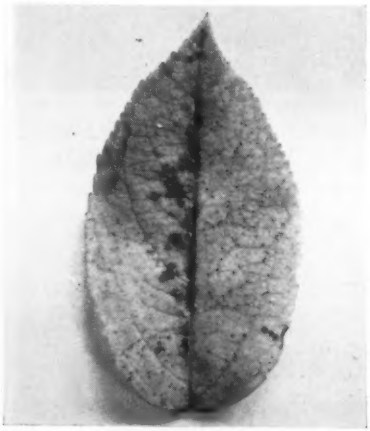


5. Portion of subcuticular growth shown by staining. × 420.

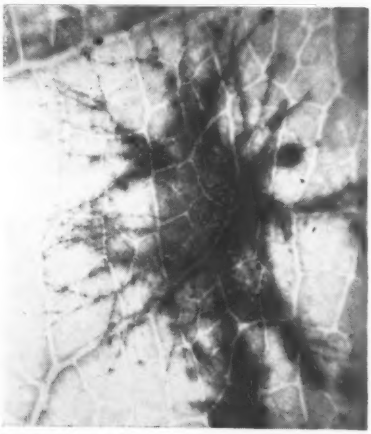
PLATE II



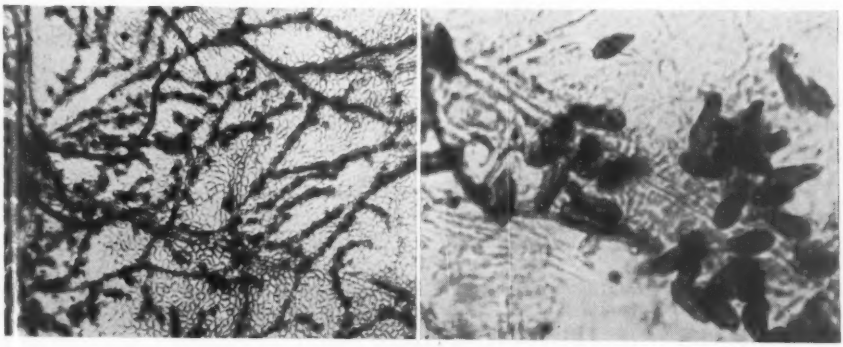
1. Apple leaves 14 days after inoculation. *Left:* Unstained. *Right:* Stained.



2 Natural infection revealed by staining.



3. One of the lesions on (1) above. × 15.



4. Detached scabbed epidermis after staining. *Left:* × 50. *Right:* × 420.

PLATE III

1. Chrysanthemum vein mottle: systemic mottle, crinkle and necrosis.

2. Tomato bushy stunt: local necrotic lesions, systemic wilt and necrosis.

3. Pea mosaic: systemic chlorotic spot mottle and dwarfing.

4. Pelargonium leaf curl: local necrotic lesions, systemic crinkle and necrotic spots.

PLATE IV

NICOTIANA CLEVELANDII GRAY AS A TEST PLANT FOR PLANT VIRUSES

by M. HOLLINGS*

Plant Pathology Laboratory, Harpenden, Herts

MANY species in the family Solanaceae have proved useful indicator hosts for many different plant viruses. *Nicotiana clevelandii* Gray, however, has received little attention, and very few references to it have done more than record it as susceptible to infection.

Tests in this laboratory during the past two and a half years have indicated that *N. clevelandii* has many advantages. It is susceptible to several viruses (for example, broad bean mottle, pea mosaic and lettuce mosaic) that are unable to infect the commonly-used members of the Solanaceae; and some others (such as chrysanthemum viruses B and vein mottle) that cause only local lesions in most species of this family, invade *N. clevelandii* systemically, reaching a high concentration. The leaves contain plenty of sap which is readily clarified and is very suitable for serological work. The plants occupy less space than tobacco for maintaining stock cultures of viruses, and most of the viruses tested could readily be isolated even from old plants. *N. clevelandii* is slightly more difficult to raise than tobacco, but remains susceptible to infection for a longer period.

Plants were raised from seed, then planted into 4½-inch-diameter pots containing John Innes No. 2 potting compost; glasshouse temperatures did not fall below 65° F. Plants were ready for use when a rosette of about six leaves had formed, and remained susceptible for several weeks—even when flowering stems had begun to grow.

The following list describes the reactions of *N. clevelandii* to mechanical inoculation with 74 isolates of 58 viruses. To increase their susceptibility plants were kept in darkness at 85° F for 24 hours before inoculation; M/15 phosphate buffer (pH 7·0) and Celite diatomaceous earth were added to the inoculum. Return inoculations to indicator plants were made from inoculated and non-inoculated leaves, to detect symptomless infections and to check the identity and concentration of the virus. Serological tests were also made, wherever possible, with sap from *N. clevelandii* to confirm the identity of the virus, and to establish the suitability of the plant for such work. Successful serological reactions are indicated by (S) in the list.

The plant remained rosetted when infected with certain viruses; this is indicated in the list by "persistent rosetting".

Anemone mosaic: pale, local necrotic spots and rings in 5–9 days; systemic chlorosis, mottle and leaf narrowing. Plant stunted. (S)

Anemone necrosis (an undescribed virus isolated from anemone roots): local, small grey necrotic spots in 3–5 days, enlarging and leading to total necrotic collapse of the inoculated leaves. Systemic necrotic speckling, wilt and death of the plant.

Beet mosaic: no local symptoms. Faint, systemic chlorotic flecks after about two weeks, developing to a uniform chlorotic mottle, with leaf distortion, some necrotic flecks, and general stunting of the plant. Previously noted by Bennett (1949).

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Beet ringspot: numerous small, local necrotic rings in 3–5 days, developing to severe whitish necrotic ring-and-line patterns on the inoculated leaves. Systemic necrotic ring-and-line patterns, dwarfing and leaf distortion, with occasional "shot-holes" in the leaf laminae. Subsequent chronic symptoms much less severe. (S)

Beet yellows: no infection by mechanical inoculation, but aphid transfers (about ten *Myzus persicae* (Sulz.) per plant) readily caused infection. Symptoms included chlorosis, leaf puckering and distortion, and persistent rosetting.

Broad bean mottle: no local symptoms; systemic chlorotic spot-mottle developing in 2–3 weeks, with slight leaf narrowing and reduction in vigour. (S)

Cabbage black ringspot: faint, local semi-necrotic spots, followed by total necrosis of the inoculated leaves. Systemic chlorotic mottle, leaf twisting, distortion, blistering, with severe stunting of the plant.

Carnation mottle: very few, faint local chlorotic spots after about two weeks, or no symptoms; virus present in very low concentration in the inoculated leaves only.

Carnation ringspot: local, sunken grey-brown irregular necrotic spots after 4–6 days, extending to cause total necrosis of the inoculated leaves. Systemic grey-brown necrotic areas on the stem and young leaves, often killing the plant in winter. (S)

Carnation vein mottle: most inoculations failed to infect; local infection without symptoms occurred with one plant, but the virus was recovered in very low concentration only.

Celery yellow vein (a soil-borne ringspot virus isolated from celery): irregular, local necrotic ring-and-line patterns in about five days; systemic mottle and slight leaf dwarfing. Later growth almost symptomless, though virus present. (S)

Chrysanthemum vein mottle: no local symptoms. Systemic chlorotic mottle in 2–4 weeks, frequently with crinkle and leaf distortion; some isolates also caused necrotic spots and areas (Plate IV, 1). Subsequent chronic symptoms less severe, with a more general chlorosis and slight mottle.

Chrysanthemum virus B (*strain 1;* Hollings, 1957): no local symptoms; slight systemic chlorotic mottle, flecks and spots in 2–4 weeks.

Cucumber mosaic (*anemone strain*): no local symptoms; slight systemic chlorosis and faint mottle after 2–3 weeks. Subsequent young growth almost symptomless.

Cucumber mosaic (*Price's yellow strain*): systemic, yellow chlorosis after 1–2 weeks, developing to a yellow mottle, with apical dwarfing and some distortion. Dark green blisters frequently occurred on subsequent growth.

Cucumber mosaic (*tomato strain*): systemic, broad chlorotic mottle developing in 1–2 weeks, with leaf twisting, some distortion, and slight dwarfing. Infected plants continued to show symptoms for several months.

Cucumber stunt mottle (an undescribed virus isolated from cucumber): irregular, local grey necrotic spots and rings after about eight days; systemic chlorotic speckle-mottle, with fine necrotic rings, flecks and line patterns. Subsequent chronic symptoms much less severe.

Cymbidium mosaic: faint, local grey-green spots in 7–10 days; no systemic symptoms, but the virus was recovered from uninoculated leaves on one occasion only.

Henbane mosaic: no local symptoms; slight systemic vein clearing in 1–2 weeks, developing to a slight chlorotic mottle and dwarfing.

Lettuce mosaic: local chlorotic spots and rings, sometimes absent; slight systemic chlorotic flecks and spots after 2–3 weeks, developing to a general chlorotic spot mottle, with few chlorotic rings. Plants slightly dwarfed. This has previously been reported by Costa and Duffus (1958).

Lucerne mosaic: pale, local semi-necrotic small rings after about six days; severe systemic speckle-mottle, with dwarfing, leaf puckering and distortion. Subsequent chronic symptoms less severe. (S)

Narcissus virus (associated with, but not proved to be the cause of, yellow stripe disease): local, symptomless; sometimes very faint local diffuse chlorotic spots.

Pea mosaic: systemic chlorotic spot and fleck mottle, developing slowly in 2–4 weeks; growth reduced. (Plate IV, 3.)

Pelargonium leaf curl (*two strains*)*:* both strains caused local, chlorotic and semi-necrotic spots and rings in 4–9 days, followed by systemic chlorotic and necrotic rings, flecks and spots and, usually, a characteristic necrotic crinkling of the younger leaves. (Plate IV, 4.) Young plants frequently killed in winter. (S)

Potato leaf roll: no infection by mechanical inoculation; aphid transfer (using about ten *Myzus persicae* per plant) readily caused infection. Symptoms were very slight—a faint chlorosis, which was not observed in plants infected in winter.

Potato paracrinkle: slight systemic chlorotic mottle in 2–3 weeks, becoming more severe, with dwarfing of the plant and buckling of the leaves. (S)

Potato veinal necrosis: occasionally, few local chlorotic spots, but usually no local symptoms. Systemic symptoms very similar to those of virus Y.

Potato virus A: slight systemic chlorosis, faint mottle and leaf bending, with patches of yellow banding along some of the larger veins. Plants dwarfed slightly.

Potato virus C (two strains)*:* one strain caused faint local chlorotic spots in three days, followed by systemic apical vein clearing, developing to a severe mottle with necrotic speckling, twisting, down-curling and crinkling of the younger leaves, and persistent rosetting. The plant was usually killed within 3–4 weeks. A second strain caused numerous small local necrotic dots after three days, followed by systemic symptoms similar to those of the first strain, but lacking the down-curling of the leaves. (S)

Potato virus G (*aucuba mosaic*)*:* local, semi-necrotic spots and flecks after 3–6 days, the lesions coalescing to form necrotic areas. Systemic necrotic spots and areas, with stunted growth. Subsequent chronic symptoms only slight—faint grey-green mottle and narrowing of the leaves.

Potato virus X (*two strains*)*:* a strain causing only slight symptoms in potato and tobacco caused few, faint local chlorotic spots in about five days, followed by systemic chlorosis and faint pale green mottle. A more virulent strain produced more severe symptoms in *N. clevelandii*, with considerable necrosis and dwarfing. (S)

Potato virus Y: faint local chlorotic spots, sometimes absent. Systemic fine yellow-netting of the smaller veins, developing to a severe vein chlorosis, twisting, dwarfing and persistent rosetting of the younger growth, and killing the plant within three weeks in winter. (S)

Raspberry ringspot: numerous, local grey spots after 5–7 days, developing to necrotic rings and ringspots. Systemic pale necrotic speckles and small rings, with much leaf buckling and distortion. Subsequent chronic symptoms less severe: a fine speckle-mottle, with few necrotic areas.

Raspberry yellow dwarf: numerous, pale local semi-necrotic small spots after five days. Systemic chlorosis, mottle, with some rings and flecks after 1–2 weeks; plants dwarfed, with slight leaf distortion and asymmetry. (S)

Tobacco mosaic: several isolates from tomato produced local sunken brown necrotic dots, similar to those in *N. glutinosa*, in 2–3 days; these enlarged to brown-rimmed lesions. A strain of TMV from tomato designated "tomato black fleck strain" caused local brown or black ring lesions in 4–5 days, followed by systemic crinkle, mottle and dwarfing. (S)

Tobacco necrosis: numerous, small sunken grey-brown necrotic local lesions, rather similar to those of tobacco mosaic, after two days. The lesions coalesced, causing total necrosis of the inoculated leaves. (S). Plants infected from the soil sometimes became systemically infected, though symptomless.

Tobacco rattle: no local symptoms; fine systemic speckle-mottle in about seven days, followed by puckering, chlorosis and severe dwarfing of the leaves, and persistent rosetting.

Tobacco ringspot: numerous, local sunken grey-brown spots in about four days; local lesions enlarge, causing total necrosis of the inoculated leaves. Systemic necrotic dots and speckling, with some stunting of the plant, and frequently necrosis and bending of the leaves. Plants killed within two weeks in winter.

Tobacco severe etch: indefinite, local chlorotic spots after about three days; systemic yellow-netting of the smaller veins of the apical leaves, developing to a severe mottle, with dwarfing, chlorosis and persistent rosetting.

Tomato aspermy (*strains 1 and 2;* Hollings, 1955): few, faint local chlorotic spots in about five days. Systemic speckle-mottle, with apical dwarfing and persistent rosetting. Plant killed in 3–4 weeks in winter.

Tomato black ring: numerous small, pale semi-necrotic local rings and flecks in three days; slight systemic mottle, with irregular rings, flecks and line patterns. Slight dwarfing. Subsequent chronic symptoms very slight or none. (S)

Tomato bushy stunt: local grey-brown necrotic spots in about six days; systemic chlorosis, wilting, and necrosis, killing the plant in winter (Plate IV, 2).

Tomato spotted wilt (*four isolates*)*:* faint whitish local rings in 4–5 days, often becoming oily, concentric necrotic rings. Systemic necrosis which, with virulent isolates, killed the plants.

No infection was obtained with any of the following viruses: abutilon infectious variegation*, barley stripe mosaic, bean yellow mosaic, chrysanthemum virus D, chrysanthemum virus E*, chrysanthemum ring pattern, chrysanthemum stunt, cauliflower mosaic, cocksfoot streak, cucumber green mottle, dahlia mosaic, hydrangea ringspot, laburnum mosaic*, pelargonium ringspot*, potato virus S (Arran Victory strain), turnip crinkle or turnip yellow mosaic (Newcastle strain). Except for the four viruses marked *, all have proved mechanically transmissible to other host plants.

SUMMARY

Nicotiana clevelandii Gray, which had previously received little attention as a test plant, was susceptible to infection by 41 of 58 plant viruses tested. The plant proved a useful indicator for some, and a good virus source for many more, including several for which no other Solanaceous host plant has been found (broad bean mottle, pea mosaic, lettuce mosaic and a narcissus virus). Sap from this plant was readily clarified, and proved suitable for serological work.

I should like to thank Miss Diana M. Stacy, of Plant Pathology Laboratory, for valuable technical help.

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FRIT FLY IN SPRING OATS

III. SAMPLING FOR SECOND GENERATION GRAIN ATTACK

Contribution from the Conference of Advisory Entomologists, National Agricultural Advisory Service, summarized by A. H. Strickland, of the Plant Pathology Laboratory, Harpenden, Herts

THIS article presents the grain-sampling results obtained in the Advisory Entomologists' experimental plot work on frit fly during 1953, 1954, and 1955.

Before the work was started the results of Cunliffe and Fryer (1924) on the relative infestation of main and bosom grains within the oat panicle were considered. On the advice of D. W. Empson—who has since published his results (Empson, 1957, 1957a)—it was decided that sub-samples of fifty main and fifty bosom grains should be dissected to assess percentage grain damage on each trial plot. The results in this paper refer to such sub-samples, and to trials at the following localities:

1953: Long Marston and Oulton, Yorks; Saredon, Staffs; Gleadthorpe, Notts; Trumpington, Cambs; Harlow, Essex; Wantage, Berks; Kingsnorth, Kent; Starcross, Devon; Chainbridge, Monmouth; Llwynfilly, Brecon; Glangwy, Radnor; and Penmount, Caerns.

1954: Methley, Yorks; Detton, Salop; Thaxted, Essex; Hinxhill, Kent; Wickwar, Glos; Powderham, Devon; Llangorse, Brecon; Rhuddlan, Flints; and Gleadthorpe, Wantage, Chainbridge, and Glangwy.

1955: Longbenton, Northumberland; Wike, Yorks; Rodbaston, Staffs; Clifton Bury, Beds; Bethersden, Kent; Tredomen Court, Brecon; and Methley, Gleadthorpe, Wantage, Powderham, Chainbridge, and Rhuddlan.

SAMPLING RATES

In the work referred to above, Cunliffe and Fryer dissected six sub-samples of grain from bulked harvest samples of each of the oat varieties in which they were interested. The numbers of grains varied: Cunliffe's percentages at Oxford were based on sub-samples of about 111 grains each, while Fryer, at Harpenden, dissected an average of 134 per sub-sample. Their combined results over twelve oat varieties gave average percentage infestations of 36·0 (Oxford) and 17·4 (Harpenden), the coefficients of variation being about 7 and 6 per cent respectively.

These data were considered in the light of the results from the Advisory Entomologists' Extensive Survey (Thomas, 1954) which showed that, over the period 1947–51, grain damage to commercial crops in England and Wales averaged about 8 per cent.

Cunliffe and Fryer's results were not considered to give more than a rough idea of the sampling errors to be expected in grain dissection. Fryer's trials were, for example, done in a garden in the middle of Harpenden, while the late drilling (April 18) at Oxford and Harpenden resulted in much heavier grain damage than might be expected on plots set up within commercial oat crops scattered over England and Wales, and drilled somewhat earlier.

To check on the sampling errors over what was anticipated would be a wide range of infestation levels, an intensive grain dissection routine was specified for the first (1953) season of the Advisers' co-operative trials. Five "grab" samples of panicles were taken at random on each plot, and to avoid sub-conscious selection of panicles on abnormally long or short stems it was specified that the grabs were to be taken by bending down and grasping a handful of stalks just above ground level. The grain in each grab was rubbed out, coned and quartered, and two sub-samples, each of fifty main and fifty bosom grains, were taken for dissection, giving plot means based on 1,000 grains, and treatment means based on 4,000.

This routine was adopted at eight trial sites (Staffs, Yorks, Notts, Essex, Kent, Devon, and Caerns.) in 1953, and the results were analysed after angular transformation of the infestation percentages.

Attack over all treatments at these eight sites averaged 17·1 (= 8·6 per cent) with a variance, based on four replicates of ten samples per plot, of 2·36, giving a coefficient of variation of 9 per cent of the mean. Further analysis showed that, on a four-replicate basis, three 100-grain sub-samples per plot would give infestation estimates with errors equal to about 10 per cent of the mean over the range 3·3–44·4 (= 0·3–49·0 per cent). As an additional check, 176 within-plot variances were divided into two groups, depending on whether attack was less, or more, than 25 per cent on the individual plots. The mean of the first series was 15·4 (= 7·1 per cent), the second 40·0 (= 41·3 per cent). The two pooled variance estimates indicated that three 100-grain sub-samples would give infestation estimates with coefficients of variation of about 11 per cent at the lower, and 4 per cent at the higher, levels of damage. Errors of these magnitudes were acceptable, as can be judged by comparison with the first generation shoot sample errors discussed in an earlier paper (Strickland, 1959).

In 1954 and subsequent years, then, 300 grains (150 of each type) were dissected from a bulked sample of five panicle grabs per plot. A detailed analysis has been done on the results for 1954 and 1955; four of the 1953 trials (Cambs., Essex, Brecon, and Radnor), where time did not permit of the dissection of more than 300 grains per plot, have been included in this overall analysis, which took the form:

Factor	*Degrees of Freedom*	*Mean Square*
Between plots ..	329	27·11
Within plots	1,048	9·33

The grain-infestation data pooled in this analysis had an overall mean of 13·6 (= 5·5 per cent), the range being 1·6–35·3 (= 0·1–33·4 per cent); the standard error estimates shown in Table 1 therefore refer to the normal range of infestation levels found even in late-drilled commercial crops in England and Wales.

TABLE 1

Standard Errors Expected when Sampling for Grain Damage by Dissection

Replicates per Trial	Number of Grains Dissected in each Plot:						
	50	*100*	*200*	*300*	*500*	*1,000*	*10,000*
1	5·0	3·9	3·3	3·0	2·8	2·6	2·5
2	3·5	2·8	2·3	2·1	2·0	1·9	1·7
4	2·5	2·0	1·6	1·5	1·4	1·3	1·2
6	2·0	1·6	1·3	1·2	1·1	1·1	1·0
8	1·8	1·4	1·2	1·1	1·0	0·9	0·9
10	1·6	1·2	1·0	1·0	0·9	0·8	0·8
100	0·5	0·4	0·3	0·3	0·3	0·3	0·2

As was noted in the shoot-attack analysis referred to above, sampling error can be reduced most economically by increasing replication of plots, rather than by dissecting more grains within each of a small number of replicates. Dissection of 500 grains from each of four replicates gives an average infestation estimate with the same error as could be obtained by dissecting 100 grains from each of eight replicates. It is clear, from the Table, that there is little point in increasing sampling rate much above 300 grains per plot.

LOCALITY, TIME, AND INTENSITY OF ATTACK

Two analyses have been done on the available within-plot variance data. The first covered ten groups of sites over three years, and was designed to show whether there were any real differences in grain-infestation pattern between site localities and years, as was noticed with shoot attack in the first paper in this series. The rounded-off data are shown in Table 2.

It will be seen that grain attack over all sites averaged about 17 (= 8·5 per cent) in each year, though there was considerable variation between sites in any one year: in 1953 a factor of 4, in 1954 a factor of 17, and in 1955 a factor of 5, indicate the extent of this variation. It can also be seen that the average variances for each year show the same trend as the shoot variances in the earlier paper, suggesting again that 1953 was a year when attack was abnormally variable over both generations. Closer inspection of Table 2 suggests, however, that this trend was not so marked as in the shoot data: in Nottingham, Berkshire,

and Monmouth the trials were on the same farms in all three years, and in the last two localities the variances were practically identical in all years. It seemed possible that the greater 1953 variability could have been a site, a year, or an interaction effect, and to resolve the matter as far as possible the variances in Table 2 have been analysed after logarithmic transformation:

Factor	*Degrees of Freedom*	*Mean Square*
Years	2	0·109
Localities	9	0·025
Residual	18	0·040

The year-variance ratio in this analysis (2·7) indicates that average differences of the order shown in the last line of Table 2 would be obtained by chance once in every ten sets of comparisons. There is no suggestion in the analysis that grain attack was distributed in a consistently more uniform pattern on some sites compared with others and, but for the unusual variability in shoot attack in 1953, it would be fair to conclude that, since the grain variance ratio of 2·7 does not reach the conventional five per cent significance level, the high average 1953 grain variance was a chance effect. Overall, however, it seems reasonable to suggest that there are years when both first and second generation attacks develop in patches rather than uniformly over a crop. Such could result from many causes, like uneven germination giving rise to areas where crop growth is backward and attractive to egg-laying adult flies.

TABLE 2

Angular Infestation, and Variance, Estimates for Ten Sets of Trials

Locality	1953		1954		1955		Average	
	*Inf.**	*Var.**	*Inf.*	*Var.*	*Inf.*	*Var.*	*Inf.*	*Var.*
Yorks	7	14·4	22	6·1	7	11·3	12	10·6
Staffs/Salop	13	7·5	4	11·5	9	12·9	9	10·6
Notts	23	12·7	15	4·3	7	7·0	15	8·0
Essex/Beds	26	12·4	9	11·3	33	4·8	23	9·5
Kent	26	24·6	34	3·3	22	9·5	27	12·5
Berks	28	7·2	27	6·8	35	7·0	30	7·0
Devon	14	23·6	2	8·2	10	12·2	9	14·7
Monmouth	16	8·0	5	9·6	26	6·5	16	8·0
Brecon	15	7·2	13	10·0	8	17·9	12	11·7
Flints	7	15·0	28	8·6	8	13·6	15	12·4
Average	18	13·3	16	8·0	17	10·3	17	10·5

* *Inf.* = grain infestation; *Var.* = within-plot variance.

The second within-plot variance analysis was done on the 1955 results. In this year adjacent trials with the varieties Star and Sun II were set up in Northumberland, Nottingham, Kent, Devon, and Brecon. Over these sites the Star plots gave an average grain infestation of 9·9, the Sun II of 9·4 (= 3·0 and 2·7 per cent), and the average within-plot variances were 10·9 and 10·0 respectively. Clearly there was no difference in the susceptibility of the two varieties to grain damage, or in the distribution of the damage within the respective varietal blocks.

MAIN AND BOSOM GRAIN DAMAGE

In their 1924 paper, Cunliffe and Fryer gave details of main and bosom grain damage at Oxford. The overall attack was 34·4 per cent, 45·9 per cent of the main, and 22·8 per cent of the bosom, grains being damaged. Subsequently Empson (1957a) has suggested that roughly equal numbers of both types of grain are damaged at high levels of attack, while at low levels (less than 10 per cent) about four times as many main as bosom grains are damaged.

General confirmation of Empson's results (which related specifically to crops in the West Midlands) has been obtained in the co-operative work throughout England and Wales. The relevant results are shown in Table 3, alongside averages struck from the data given in Empson's paper.

TABLE 3

Ratio of Main to Bosom Grains Attacked at Three Levels of Damage

Overall Percentage of Grain Damaged	1953		1954		1955		Average M/B Ratios	
	No. Trials	M/B Ratio	No. Trials	M/B Ratio	No. Trials	M/B Ratio	N.A.A.S. Work	Empson (1957 a)
Less than 10 ..	4	5·0	6	5·9	11	5·6	5·6	4·1
10–20.. ..	1	4·4	3	2·5	2	3·6	3·2	4·0
Over 20 ..	3	2·9	3	1·8	2	2·2	2·3	2·9

Although the range of main to bosom grain ratios in the N.A.A.S. co-operative work was from 1·0 (a 64 per cent attack in Kent in 1954) to 20·3 (a 2·5 per cent attack in Brecon in 1955), considerable variation was found: ratios of 1·8 with a 5 per cent attack in 1953, and 1·9 with a 43 per cent attack in 1954, testify to the fact that there is nothing particularly rigid about the main to bosom grain ratios in Table 3. Suffice it to say that, at infestation levels likely to be met in field work on commercial crops, something like three times as many main as bosom grains will be found to be damaged.

Most of the oat varieties now grown commercially produce approximately equal numbers of main and bosom grains. A hundred grains taken at random from a bulked panicle sample after rubbing out should contain equal numbers of each grain type, and percentage infestations calculated from such sub-samples should be consistent and reproducible. In practice this ideal is not always attained, as can be seen from Table 4.

From the sampling aspect the variation in main grain proportion is of little importance. Over the three seasons for which co-operative main and bosom grain results were recorded separately 113,250 of each type were dissected in 37 experiments; 16,369 infested main, and 6,365 infested bosom, grains were found, giving a pooled average infestation of 10 per cent (main = 14·5, bosom = 5·6). On average, then, a sample of 150 of each type of grain gave 21 fritted mains and 9 fritted bosoms.

Small variations in main to bosom grain proportions would not greatly change this result. For example, had the average sample consisted of 188 main grains and only 112 bosom grains, the overall attack would have been estimated as 11,

TABLE 4

Proportions of Main Grains in Random Samples of 300 Grains

Year	Number of Trials	Main Grains per Random Sample of 300 Grains	
		Mean Number	*Range*
1954	10	161	150–188
1955	9	155	131–174
Average per trial ..	.. .. ..	158 ± 13·2	

instead of 10, per cent; while at the other end of the range shown in Table 4 (131 main and 169 bosom), the attack would have been estimated as 9·5 per cent.

Random samples of grain, from panicles which have been coned and quartered after rubbing out and winnowing, clearly give good infestation estimates, and in large-scale experimental and survey work there is no need to spend additional time ensuring that each dissection sample is precisely balanced in respect of grain type.

SUMMARY

Second generation frit attack in oat grains can be estimated by dissecting grain samples taken in the field at harvest. This method is discussed in relation to the sampling errors involved, and a Table is presented showing the standard errors to be expected over a range of field sample sizes. Normal variations in the proportions of main and bosom grains in dissection samples do not materially affect the infestation estimates obtained, though careful mixing of all grain from random samples of panicles is essential before sub-samples are taken for dissection.

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THE SUSCEPTIBILITY OF ATLE WHEAT TO ATTACK BY THE WHEAT BLOSSOM MIDGES

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ATLE, in a spring wheat variety trial in Co. Wexford, Eire, was shown by Mullin, Fox and Brady (*Nature, Lond.*, 1952, **169**, 629–30) to be significantly less attacked by *Contarinia tritici* Kirby than were Karn II, Progress and Ella. They stated that the infestation of Atle (34 per cent, as compared with Progress 59·2 and 53·4 per cent) was not smaller simply because this variety escaped infestation by not being in ear at the time of the midges' flight, since these two varieties came into ear simultaneously. They obtained confirmation of this relative resistance of Atle, as compared with Progress, from the subsequent yields on many farms, but the nature of the resistance remained unknown.

The purpose of our investigation was to examine the susceptibility of Atle to attack by both the wheat blossom midges, *C. tritici* Kirby and *Sitodiplosis mosellana* Géhin.

In 1958, four rows (two sown on each of two dates) of each of three spring wheats, Atle, Svenno and Fylgia, and two winter wheats, Hybrid 46 and Squarehead's Master, were grown at Rothamsted Lodge. Each morning (June 18–July 14) counts were made of the numbers of ears visible, and each evening (June 18–July 11) of the numbers of midges seen ovipositing. The number of *S. mosellana* on the plot was increased by occasionally liberating individuals that had emerged in the insectary. The actual infestations were later determined by examining 100 (4 × 25) ears of each variety of spring wheat. Information on the dates of the midges' emergence in 1958 was provided by the routine daily observations on the numbers emerging in the insectary.

The two winter wheats were sown late and did not ear until after the main flight of the midges, so they will not be considered further.

The spring wheats came into ear when the midges were on the wing and could show preference in their oviposition. On the twenty-four evenings (two were too wet) on which the ovipositing midges were counted, 717 *Contarinia tritici* were seen on Atle, 881 on Svenno and 372 on Fylgia, indicating that Atle was not appreciably less attractive than Svenno. The fewer midges on Fylgia could be accounted for by the fact that its ear emerged slightly later than those of Atle and Svenno. Very few *Sitodiplosis mosellana* were seen ovipositing; probably the ears were examined too early in the evening, because this species starts ovipositing later than *Contarinia tritici*.

Table 1 shows the infestation on July 16 by *C. tritici* (T) and *Sitodiplosis mosellana* (M). The figures for the average number of larvae per attacked grain indicate that the sampling was done at the correct date, i.e., before the larvae had started descending from the ears.

Contarinia tritici was also noticed ovipositing apparently as freely on Atle as on the other varieties in the wheat plots at the National Agricultural Advisory Service regional headquarters at Cambridge on June 20. The infestation on July 11 was so high that only ten ears of each variety were examined in detail

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TABLE 1

Infestation of Atle, Svenno and Fylgia at Rothamsted Lodge on July 16, 1958, by the Wheat Blossom Midges

	Total Larvae in 100 Ears		Percentage Grain Attack		Average Number of Larvae per Attacked Grain	
	T	M	T	M	T	M
Atle	2,209	461	5·2	7·3	10·7	1·6
Svenno	1,589	368	3·6	5·8	11·4	1·6
Fylgia	1,351	359	3·4	6·2	10·7	1·5

(Table 2). These differences in infestation between the varieties could probably be accounted for by slight differences in the correlation of ear emergence and midge emergence.

TABLE 2

Infestation of Atle, Svenno, Peko, Koga II and Ritchie at Cambridge on July 11, 1958, by the Wheat Blossom Midges

	Total Larvae in 10 Ears		Percentage Grain Attack		Average Number of Larvae per Attacked Grain	
	T	M	T	M	T	M
Atle	1,486	95	24·5	12·1	11·5	1·5
Svenno	559	48	11·4	7·8	10·9	1·4
Peko	770	120	13·3	26·6	12·8	1·6
Koga II	919	224	17·0	29·5	10·7	1·5
Ritchie	374	122	7·6	17·6	10·7	1·5

SUMMARY

Observations in 1958 on rows of wheat varieties at Harpenden and Cambridge indicate that Atle, under certain conditions and levels of infestation, is no less attractive to ovipositing wheat blossom midges than is Svenno and some other spring varieties. The larvae of these midges also develop equally well on Atle.

We are indebted to Mr. E. G. Thompson for supplying us with the wheat seeds, and to Mr. P. W. Carden for sending us the samples of infested wheats from Cambridge.

SUGAR BEET YELLOWS IN GREAT BRITAIN, 1958

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SUGAR beet yellows threatened to spread in 1958 from the many sources of virus persisting from the previous year; but wet, stormy weather in June, and extensive spraying of sugar beet crops with systemic insecticide limited its incidence. In 147 fields sampled by the agricultural staff of the British Sugar Corporation an average of 0·3, 4·2, 14·1 and 20·7 per cent of plants had yellows at the end of June, July, August and September respectively. The percentage of the entire crop of 413,700 acres with under 1 per cent of infected plants at the end of August was 20·3; with 1–20 per cent, 58·3; with 21–60 per cent, 17·3; and with over 60 per cent, 4·1. Few crops were uniformly yellow, and infected plants were generally in patches.

As usual, yellows incidence at the end of September was highest in Suffolk, Essex and south Cambridgeshire (30–50 per cent), and least in Yorkshire and Scotland (1–4 per cent). It was relatively low in west Norfolk and south Lincolnshire (8–19 per cent), and relatively high in the Midlands and west (24–31 per cent).

Yellows caused the following calculated losses in 1958 (cf. Hull, *Plant Pathology*, 1953, **2,** 39–43; 1954, **3,** 130; 1955, **4,** 134; 1956, **5,** 130; 1957, **6,** 131; 1958, **7,** 131):

Calculated Percentage loss of Sugar Yield		Actual Yield per Acre		Estimated Potential Yield of Roots per Acre*	Estimated Loss of Root Yield*
		Sugar	Roots*		
A†	C†	*tons*	*tons*	*tons*	*tons* (000)
5·8	7·7	2·09	13·08	13·89	336

* Converted to equivalent yields at a sugar content of 16 per cent.

† A: Assuming 4¼ per cent loss for each week the plants show symptoms. C: Assuming 2·15 cwt per acre loss for each 10 per cent infected plants at the end of August. (Calculated from yellows incidence and factory yields, 1946–57.)

Water-logged soil in July made the leaves of many sugar beet crops turn yellow, but affected crops made a remarkable recovery by the end of the summer and appeared to suffer little decrease of yield. The foliage of most crops was rank, and root yields increased more than usual during October and November. The average yield of roots (13·9 tons per acre) was the heaviest recorded in Great Britain, but the sugar content was low (15·08 per cent) and the yield of sugar (2·09 cwt per acre) was exceeded in 1950 (2·12 cwt per acre) and 1953 (2·14 cwt per acre), and almost equalled in 1956 (2·04 cwt per acre). The estimated potential yield of roots at 16 per cent sugar content in the absence of yellows has been exceeded only in 1950 (14·01 tons per acre), but was almost paralleled in 1953 (13·76 tons per acre) when estimated loss from yellows was about half that in 1958.

Most growers in the eastern counties were advised to spray their crops with systemic insecticide during May or June on the basis of the Sugar Corporation's daily counts of aphids on sugar beet. Wet weather prevented spraying in some areas, but about 100,000 acres of sugar beet were sprayed. At the end of September the incidence of yellows was, on average, 2½ times more in unsprayed fields than in sprayed ones in the same locality. Experiments showed that a single spraying increased yields by 7–8 per cent, on average, in crops with yellows.

SOIL SAMPLING PROCEDURES FOR POTATO ROOT EELWORM CYSTS

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ANSCOMBE (1950) and Jones (1955) have pointed out that there is little information on which to base satisfactory sampling methods for assessing field populations of potato root eelworm, *Heterodera rostochiensis* Woll. In 1957, the N.A.A.S. Conference of Advisory Entomologists set up a working party consisting of D. W. Fenwick, J. H. Fidler, F. G. W. Jones, H. W. Thompson and ourselves to study this question, particularly in relation to sampling for advisory purposes.

The principles of soil sampling for *Heterodera* species have been discussed by Fenwick (1959), and Fidler, Church and Southey (1959), in addition to the authors already quoted. The main need was for further information on field heterogeneity of potato root eelworm populations, and a co-operative experiment was, therefore, carried out by entomologists in different parts of the country. Twelve fields known to contain potato root eelworm populations near the level that might lead to crop damage were sampled in the following way.

Using a 1-inch diameter auger or cheese corer, 100 individual samples were taken at the points of a 10 × 10 square grid with the sampling points 15 yards apart. To ensure that enough soil was available, two cores were taken at each point and bulked. The soil from each sample point was dried, sieved and mixed and a sub-sample of 50 g floated in a Fenwick apparatus (Fenwick, 1940). Total cysts and those containing fifty or more eggs (referred to as "full cysts") were counted.

Analyses of variance were carried out on the square roots of the full cyst counts, obtaining estimates of the variance within the entire areas covered by the grids (4·6 acres), within quarters of the grids (1·1 acres) and within areas covered by groups of four adjacent sampling points (0·2 acres). Table 1 shows that some fields were considerably more heterogeneous than others, but the square root transformation has eliminated any strong association between average cyst counts and variance estimates. Average variance estimates for these fields may therefore be used as a guide to the likely sampling errors in fields with cyst populations in the range 5–100 cysts per 100 g.

The average within-field variance of the square root of the cyst count per 50 g is 2·9; this is an estimate of average sampling error for a determination of field cyst population from 50 g soil taken from one randomly located point. The average variances for sampling within different areas are plotted in Fig. 1. The variances increase rapidly with acreage up to 1–2 acres and more slowly for larger areas. Thus, the average variance for fields of 5–10 acres which have been uniformly cropped and treated is probably not much greater than that estimated for areas of 4·6 acres.

Fig. 1 suggests that the variance between 50 g samples taken from points only a yard or so apart is about 1·2, which is much the same as that found by

Fidler *et al.*, (1959) for cereal root eelworm. It is, therefore, reasonable to assume that the variance between contiguous 50 g samples for potato root eelworm is about 0·72, as was taken by Fidler *et al.*, for cereal root eelworm. However, the average variance between points located at random in a field (2·9) is considerably greater than the comparable estimate of 1·7 which they obtained for cereal root eelworm. Although all potato root eelworm populations are not equally heterogeneous, this species evidently tends to be more patchily distributed in fields than cereal root eelworm.

On the basis of the variance estimates quoted above, Table 2 gives the standard errors* of cyst populations per g estimated by examining the whole of various bulk soil samples from a field with 1·0 full cysts per g. The Table shows that the number of cysts in a bulk sample made up from 25–50 random points from a field of average heterogeneity, should give a sufficiently accurate estimate of cyst population for most advisory work. Unless the number of sampling points is large, there is little advantage in taking a total of more than about 2,500 g soil. It is advisable to sample fifty rather than twenty-five points because, although the average gain is small, it may be considerable for fields such as those at Dunnington and Bridford (Table 1), which are particularly heterogeneous. In balancing increased work in the field and laboratory against the increased accuracy which it would secure, one must remember that doubling the number of samples per field and the weight of the laboratory sample by no means doubles the total work.

TABLE 1

Mean Cyst Counts and Variance Estimates for Square Roots of 50 g Counts

	Mean		Mean Squares Within Blocks of Area:†		
	Full Cysts per 50 g	*Square Root of Cyst Count*	*0·2 acres*	*1·1 acres*	*4·6 acres*
Swindon, Glos	4	1·5	1·1	1·6	1·9
Bridford, Devon..	17	3·4	3·6	4·5	5·4
Cawood, Yorks	13	3·0	2·2	3·4	3·7
Dunnington, Yorks	18	3·4	1·7	5·4	6·2
Friern, Middx	11	3·0	1·2	1·5	1·8
Midfield, E. Lothian	40	6·0	3·3	3·9	4·0
Frieston, Lincs	22	4·5	1·5	1·6	1·9
Welches Dam, I. of Ely ..	22	4·5	1·1	1·7	1·8
Wimblington, I. of Ely	29	5·1	2·2	3·8	4·0
Thirsk, Yorks	7	2·5	0·9	0·9	0·9
Hetton, Durham	8	2·5	1·1	1·9	2·0
Whitchurch, Glam	4	1·7	1·0	1·0	1·0
All sites	16	3·4	1·7	2·6	2·9

† Approximate areas covered by 4 adjacent sampling points, 25 points in a square grid and 100 points in a square grid at 15-yard spacing.

Bulk samples are not normally made up from fifty or so points located independently at random in a field, as it is more convenient to sample at regular intervals along one or more traverses. When, as for the cereal root eelworm

* These have been calculated using the assumptions listed by Fidler *et al.*, (1959), p. 33; the Table may be compared with Table 2 in that paper.

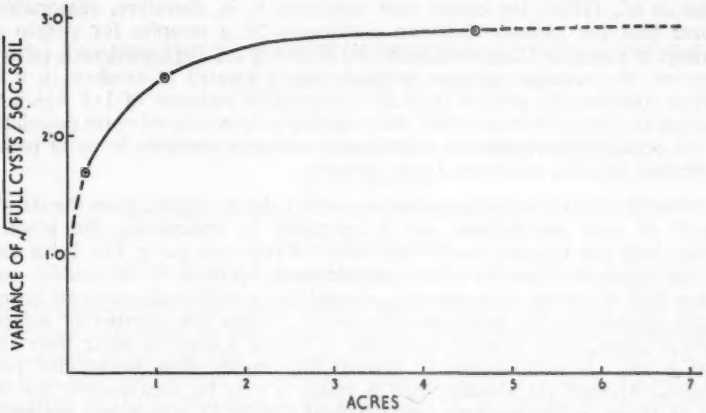

Fig. 1. Variance of cyst counts between random sampling points in different areas.

data discussed by Fidler *et al.*, the sample points are on a randomly located traverse, as in much routine field sampling, the sampling errors (which may be underestimated) must be evaluated as if the points were located independently at random. However, the present data demonstrate the advantage of locating sampling points systematically over a field in a regular grid pattern. For example, if a ten-acre field is sampled at fifty points on a regular grid, each point represents an immediate locality of about one-fifth acre; Fig. 1 shows that the average error for sampling from one-fifth acre is about 1·7 compared with 2·9 for random sampling over a field of average size. Thus, if about fifty points are sampled, the total information obtained from each point is increased by more than half, if the points are spaced in a regular grid over the field. The following Table compares the standard errors of population

TABLE 2

*Estimated Standard Errors of Full Cyst Populations per g in Bulk Soil Samples Taken from a Field with 1·0 Cysts per g**
(*whole bulk examined*)

Number of Random Sample Points (S)†	Total Weight of Bulk Soil Sample (g) (N)					
	500	*1,000*	*2,500*	*5,000*	*10,000*	*Very Large*
10	·15	·14	·14	·13	·13	·13
25	·11	·10	·09	·09	·08	·08
50	·10	·08	·07	·06	·06	·06
100	·09	·07	·05	·05	·04	·04
Very many	·08	·05	·03	·02	·02	—

* Standard errors for fields with other population densities may be estimated from the Table by multiplying by the square root of the cyst population per gramme.

† Approximately $\frac{N}{S}$ g sampled from each point.

estimates (for fields of 5–10 acres with 1·0 cysts per g) obtained by examining all the soil in bulk samples from a systematic grid of points, with those for random samples from the same number of points:

	Random Points	*Systematic Grid*
	±	±
500 g taken from 10 points	·15	·14
1,000 g taken from 25 points	·10	·08
2,500 g taken from 50 points	·07	·05
5,000 g taken from 100 points	·05	·03

Table 2 shows how accurate an estimate of cyst population per gramme can be obtained from the number of cysts in bulk soil samples obtained in different ways. However, in practice, cysts are only counted for a sub-sample from the thoroughly mixed bulk, and this introduces additional errors. Table 3 shows the effect of the amount of soil examined in the laboratory on the accuracy of population estimates obtained.

The standard errors have been calculated in the manner described by Fidler *et al.* In the absence of suitable data for potato root eelworm, the laboratory sampling errors have been assumed equal to those for cereal root eelworm, although they might be expected to be somewhat smaller.

TABLE 3

*Estimated Standard Errors of Full Cyst Populations per g Estimated by Various Sampling Procedures for a Field with 1·0 Cysts per g**

	Weight of Soil Examined in the Laboratory (g)				
	50	*100*	*200*	*400*	*Whole Bulk*
500 g taken from 10 random points	·28	·23	·19	·17	·15
1,000 g taken from 25 random points	·26	·20	·16	·13	·10
2,500 g taken from 50 random points	·25	·18	·14	·11	·07
5,000 g taken from 100 random points	·24	·18	·13	·10	·05
Large sample from many random points	·24	·17	·12	·08	—

* Standard errors for fields with other population densities may be estimated from the Table by multiplying by the square root of the cyst population per gramme.

Table 3 shows that the amount of soil examined in the laboratory is a major factor in determining the accuracy of the estimates obtained from a soil sample, and that there is a greater advantage in increasing the size of the laboratory sample if the field sample is large, and collected from many random points. Comparison of Table 3 with that given by Fidler *et al.*, shows that the differences between the estimated errors are generally small; the recommendations made in that paper for routine sampling of cereal root eelworm are, therefore, also appropriate for potato root eelworm, and probably for other *Heterodera* species.

Table 4, derived from the third line of Table 3, shows, for different soil population levels, the approximate 5 per cent and 20 per cent limits of cyst population estimates obtained by examining different amounts of soil in the laboratory. At the 5 per cent limit there is a 95 per cent chance, and at the

20 per cent limit an 80 per cent chance, that the estimate will lie within the limits given. Estimates obtained by examining 200 g soil from a bulk sample of 2,500 g from fifty points are probably sufficiently accurate for routine advisory purposes, but examination of less than 100 g is clearly inadequate, except to detect fields with heavy infestations. When the estimated population is near the "critical level" for crop damage, it may be worth examining a further 200 g from the same bulk, but usually when greater precision is needed it will be necessary to take additional bulk samples from the field.

TABLE 4

Approximate 5 per cent and 20 per cent Limits for Estimates of Full Cysts per 100 g from Laboratory Samples of Various Sizes (for bulk sample of 2,500 g from fifty random points or equivalent)

Full Cyst Population per 100 g	Weight of Soil Examined (g)			
	50	*100*	*200*	*400*
		5 per cent limits		
5	0–32	0–16	2–10	3–8
10	0–44	2–24	5–17	7–14
20	1–63	7–39	12–29	16–25
50	13–111	28–78	37–64	43–58
100	43–181	67–139	81–120	90–111
		20 per cent limits		
5	0–20	1–12	3–8	4–7
10	1–29	4–19	6–14	8–12
20	5–45	11–32	15–26	17–23
50	23–87	35–68	42–59	45–55
100	60–150	78–125	88–113	93–107

SUMMARY AND CONCLUSIONS

Analysis of the data obtained by systematic sampling of twelve fields with potato root eelworm populations near the "critical level" has provided information which should make it possible for those doing routine sampling for advisory and similar purposes to assess the accuracy of their present soil sampling methods, and to improve on them if necessary.

The field sampling errors appear to be rather greater than those observed for cereal root eelworm, but they can be significantly reduced by sampling systematically at points on a regular grid rather than at random, and much the same recommendations can be made for estimating populations of both species.

To ensure reliable results for advisory purposes it is suggested that the following conditions are necessary:—

1. Samples should be drawn from at least twenty-five, and preferably fifty, points per field or unit area up to ten acres. Larger fields should be subdivided into units of ten acres or less so that at least two or three sample points are taken per acre. The points should be systematically located on a grid, rather than on random traverses. When following population trends it will be advantageous to sample from the same points on each occasion (Jones, 1945).
2. The weight of the bulk sample removed from the field should be not less than 1,000 g, and preferably 2,500 g (the size of sampling tool being such as to ensure that twenty-five or fifty cores will provide roughly these amounts).

3. At least 100 g, and preferably 200 g, soil from each bulk sample should be examined in the laboratory, followed by a repeat determination at critical levels of infestation. Where greater precision is needed, for example, for experimental work, it would be necessary to increase the quantities sampled at every stage (that is, number of borings, weight of bulk sample, weight of laboratory sample), making determinations for two or more bulk samples to obtain some indication of the variability of the particular field.

We are indebted to J. H. Fidler, F. G. W. Jones and H. W. Thompson for advice in the preparation of this paper.

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NEW OR UNCOMMON PLANT DISEASES AND PESTS

***Elachiptera cornuta* Damaging Gladioli.** In September 1955, I recorded larvae of *Elachiptera cornuta* (Fall.) damaging gladioli at Ballater, Aberdeenshire (*Plant Pathology*, 1955, **4**, 110). Recently, Dr. H. Pape, Bielefeld, Germany, brought to my notice the first record of this species causing economic damage to gladioli. In Sandhack (*Dahlien und Gladiolen*, Berlin, 1927, 261–2), Pape recorded an instance, near Berlin in the summer of 1926, of this species causing 10 per cent damage within three beds of gladioli containing a total of 600 plants. Since my record, Böhm (*Der Pflanzenarzt*, 1957, **10**, 115) records injury, in August 1957, from Heidenreichstein in lower Austria. In view of the difficulty in obtaining Sandhack's book, Dr Pape sent me a copy of the relevant extract, and it appears that the above records of this species on gladioli are the only ones in Western Europe. Where samples of gladioli are received for examination consequent on poor growth, the possibility of damage by this species would appear to be worth investigation.

M. W. SHAW

***Anobium punctatum* Damaging *Clematis montana*.** In March 1959, I received some pieces of stem of *Clematis montana* DC., upon the surface of which were several flight holes of a wood-boring species. The shrub was growing on an east-facing house wall at Banchory, Kincardineshire, and was estimated to be about twenty years old. The main stem at the base showed large numbers of flight holes, and further samples were taken higher up to avoid complete destruction of the shrub. On opening the stems extensive larval feeding was apparent, and larvae found were identified as *Anobium punctatum* (Deg.) from Parkin's key (*Bull. ent. Res.*, 1933, **24**, 65). Although commonly encountered, it does not appear to have been recorded previously from *Clematis* spp. Despite the extensive damage, the plant produced a good display of blooms during 1959. I am grateful to Dr. E. A. Parkin for confirming the larval identification. An adult of the species has since emerged from the material collected.

M. W. SHAW

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Eelworms on Strawberries. Both *Aphelenchoides ritzema-bosi* (Schwartz) and *A. fragariae* (Ritzema Bos) are known to attack strawberries in Britain (Franklin, Mary T., *Ann. appl. Biol.*, 1950, **37**, 1–10). In the eastern counties, *A. ritzema-bosi* is common and *A. fragariae* is rarely encountered. *A. ritzema-bosi* is usually found feeding ectoparasitically on strawberry, and bunches of eelworms can often be found on folded, or partly expanded, leaves at the base of the midvein. As these leaves expand, the greyish feeding areas become visible. In 1958, on four holdings, *A. ritzema-bosi* was found living endoparasitically in strawberry leaves both under glass and in the field, although ectoparasitic damage was usually present on the same plants. Leaf invasion appeared to occur mainly on the older leaves. Symptoms took the form of dark brown blotches along the midvein or sometimes of dark green or brown blotches scattered over a yellow senescing leaf, giving a patchwork effect. These affected areas were clearly delimited by the veins and contained numerous eelworms. Endoparasitic damage has previously been recorded on strawberry by Franklin (*loc. cit.*), but the present observations suggest that it may be quite a common occurrence, which is often overlooked. The photograph (Plate I, 2) was taken by Mr. W. E. Dant.

On several occasions during the last few years, strawberry leaves showing crinkling typical of that caused by *Ditylenchus dipsaci* (Kühn) Filipjev, but often without shortening of the petioles, have been examined. It has often been impossible to find more than one or two specimens of *D. dipsaci* in the petioles and midveins of such leaves. It is fairly certain that these small numbers of eelworms are responsible for the leaf distortion but, as eggs are not always present, it is possible they belong to a race of *D. dipsaci* for which strawberry is a poor host.

E. B. BROWN

New Host Plants of *Aphelenchoides ritzema-bosi*. This eelworm was recorded in 1958 on three species of *Peperomia: P. griseo-argentea* Yuncker, *P. caperata* Yuncker (small- and large-leaved varieties) and *P. glabella* A. Dietr. (variegated). In the early stages of attack, brown spots, mostly seen on the underside of the leaves, appear. Later typical interveinal necrosis develops, the affected areas becoming yellow and water-soaked and finally dark brown. The attacks on *P. griseo-argentea* (the *P. hederaefolia* of commerce) and *P. caperata* are new host records, but *P. glabella* has been recorded as a host in Denmark by Lindhardt (*Gartnertidende*, 1955, **71**, 568–9).

E. B. BROWN

Hosts of *Erwinia amylovora* (Burr.) Winslow. Fire blight was first identified in this country in 1958 in pear orchards in Kent. In 1959, it was also confirmed on hawthorn (*Crataegus* spp.) in Kent, Surrey, the southern and south-eastern suburbs of London and at Southend on Sea, Essex; on whitebeam (*Sorbus aria* Crantz) used for street lining in Southend on Sea, Essex, in Croydon, Surrey, and in the Kent suburbs of London; on rowan (*Sorbus aucuparia* L.) in Croydon; and on *Cotoneaster salicifolia* Franch. in Kent, Surrey, and central and south-eastern London. It has also been found on other species of *Cotoneaster* including *C. melanocarpa* Lodd and *C. polyanthema* Wolf in south-east London. The disease was confirmed in these hosts by the isolation, in pure culture, of the pathogen which was identified by colony morphology, lysis by specific 'phage, agglutination with specific antiserum and pathogenicity to pear fruits. All these plants have been reported as hosts of *E. amylovora* in other countries except *S. aria*, *C. melanocarpa* and *C. polyanthema* which do not appear to have been previously recorded as hosts.

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